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THE UNIVERSITY OF ALBERTA

PHENOTYPIC AND MOLECULAR GENETIC CHARACTERIZATION OF MICE SELECTED FOR HIGH BODY WEIGHT

by

R. KEITH SALMON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

ANIMAL GENETICS

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1987

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ISBN 0-315-40858-8

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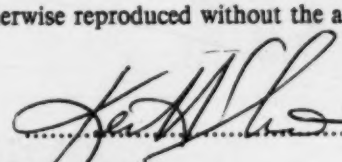
(SIGNED) 

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled PHENOTYPIC AND MOLECULAR GENETIC CHARACTERIZATION OF MICE SELECTED FOR HIGH BODY WEIGHT submitted by R. KEITH SALMON in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL GENETICS.

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Supervisor

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External Examiner

Date Sept. 14/1987

To Ruth - if all the world had such a friend ....

SPIRIT SONG

From moss burdened branches
they rise as one
Once once again
above the trees
toward the sun
They command their power
their wings
their gift
to embrace the wind
to raise them far beyond
this mist that hides the promise
alive in early dawn

And gazing upon them
we recognize ourselves
And gazing upon them
our spirits soar

In pursuit no longer
of those fleeting radiant beams
that pierce the forest dimness
yet light so little of one's dreams
They soar on thermals
born of a oneness
And their wings sing
in synchrony so true
of strength together
of giving
of rising forever
with their sun set in a world
of timeless morning blue

And gazing upon them
we recognize our strength
And gazing upon them
our spirits soar

Now unclear to earthbound eyes
images circling
just silhouettes around the sun
while their spirits seek its center
And the sun radiates praise
in shafts of brilliant white
And the praise
completes the circle
creates new life
as the eagles become one
at the center

And gazing upon them
we rise as one
And gazing upon them
our spirit soars

K. Salmon, 1985

ABSTRACT

Adipose tissue growth and lipid accumulation were investigated in both a line of mice selected for high 42 day body weight (HL) and an unselected population (FP). HL mice exhibited an accelerated growth rate, and reached a higher mature body weight, than FP mice. Relative to both body weight and nonadipose tissue weight, adipose tissue and extractable lipid increased relatively slower in HL mice than in FP mice. Only at fixed body and nonadipose tissue weights, which exceeded the maximum weights achieved by FP mice, did HL adipose tissue and lipid weights exceed those of FP mice.

In a second study, over a range of body weights lower than those examined in the first study, HL mice had more lipid, less protein and less ash than FP or HS (an additional growth-selected line) mice of the same sex and body weight. However, HL lipid accumulation (relative to body weight increase) was not accelerated in comparison to that of FP mice.

The 'high-growth' selection pressure had little influence upon the developmental relationships among five adipose tissue depots within HL mice. Relative to FP males, HL males exhibited an increase in kidney depot fat weight and an associated decrease in hindlimb depot fat weight. Within females, no obvious line differences existed in either the partitioning or the distribution of fat.

Restriction site analysis revealed a variant growth hormone (GH) gene haplotype within HL mice. Relative to the FP haplotype, the HL haplotype exhibited restriction fragment length polymorphisms for each of seven different restriction enzymes. Three of the polymorphic sites lie within 1.1 kb of the 5' end of the structural gene; a fourth polymorphism exists within the structural gene.

Changes in pituitary GH mRNA pool size were characterized throughout the early postnatal growth of HL and FP mice. While both sexes exhibited similar age-related GH mRNA profiles, males achieved and maintained larger GH mRNA pool sizes than females. There is little evidence that the involvement of the HL GH haplotype in rapid growth is mediated via the enhancement of the GH mRNA pool size in HL mice.

ACKNOWLEDGEMENTS

We are born with such a strong, pure connection to our spirituality - a connection that allows infinite potential and personal power. Unfortunately, as children, we seem unaware of its value - many of us allow the myriad of social pressures and expectations to short-circuit this connection. From such an early age, we are taught to evaluate our performance in work, in play, in life itself, on the basis of how well we have fulfilled the expectations of others. Often, we reach a stage of seldom trusting our own feelings. As a consequence, many of us come to feel that we have no personal control over our lives; we become afraid to trust and act upon our intuition - we simply react as our lives unfold around us. I, therefore, believe that the most wondrous gifts that we can receive from our parents are freedom and trust: freedom to choose our own unique journey through life; and trust that we know exactly what is right for that journey. Such freedom and trust can only strengthen a child's sense of personal power and spirituality. My parents, Ken and Joan Salmon, gave me these gifts - and there are no gifts that I will ever value more.

Their gifts of freedom and trust have allowed me to experience so many incredible things. One of the most incredible experiences has been my relationship with my advisor, Roy Berg. I have never specifically discussed my concepts of spirituality and personal power with him - there seemed to be no need. I have always sensed in him tremendous inner strength. This strength allowed him to give me more freedom and trust than I was, at times, willing to give my self. During a period of my Ph.D. program when I experienced tremendous self-doubt, Roy always trusted that I was taking the path that was right for me. Over the years, he has given me so much - but it is his trust and his friendship that I will value forever.

Roy's trust in me allowed me to venture beyond the confines of the Animal Science Department during my thesis work; I spent a great deal of time working in the laboratory of Ross Hodgetts in the Genetics Department. He and his people - Dan, Bill, Charlotte, Adam and on and on - helped me so much and I learned so much.

While I have not specifically cited their work in my thesis, the philosophies of Joseph Murphy, Shakti Gawain and Dr. A.G. Scott have influenced every aspect of my life - including the way that I approach science. I hope that I may give my students what these people have given me.

And there are so many others who have guided me - I'm sure they know who they are.

And then there is Sophie - I know no one else who maintains her/his spirituality so effortlessly.

And then there has always, always been Ruth

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I. INTRODUCTION

Mankind's awareness of the power of artificial selection pressure, to 'mold' the phenotypic norm of his domestic populations, began with his first attempts at domestication and, thereby, pre-dates written history. However, scientific consideration of the power of selection pressure to mediate phenotypic change was limited prior to the 1856 presentation of the concepts propounded by Charles Darwin (and Alfred Wallace). Darwin observed that phenotypic variability appeared in domesticated populations with each new generation. Yet by allowing only those animals with the most desirable phenotype to contribute to the next generation, the phenotypic norm for the population could be gradually shifted in a specific, and predictable, direction (Darwin, 1856).

Darwin did not, however, understand the source of his observed phenotypic variability. He could, thus, not speculate on the mechanisms which allowed a population's phenotypic norm to be molded or shifted. However, his establishment of the dynamic relationship between the phenotypic norm of a population, and the imposed selection pressures, laid the foundation for a genetic interpretation of selection theory - an interpretation that began with the reconsideration of Gregor Mendel's work in 1900 (Mayr, 1978). The tremendous expansion in the understanding of gene structure/function, which grew from Mendel's 'hereditary units', allowed population geneticists to speculate that phenotypic variability was due to the existence of functionally different forms (alleles) of genes regulating the manifestation of the phenotype (Clarke, 1975). From this speculation grew the concept that selection pressure mediates phenotypic change by indirectly increasing the frequency of alleles (and allelic combinations) that make a positive contribution to the desired phenotype (Mayr, 1978).

The electrophoretic separation of protein variants, as well as the identification of amino acid substitutions via peptide sequencing technology, provided support for this concept: variation at the protein level theoretically reflects variability at the gene level; moreover, if the protein variants function differently, then these functional differences would

be responsible for the phenotypic variability that exists within a population.

While the genetic aspects of selection theory received scientific support as a result of the identification of protein polymorphisms, an evaluation of the theory at the molecular genetic level was not possible prior to the recent advent of recombinant DNA technology. Recombinant DNA techniques offer the potential to characterize the selection-mediated molecular genetic changes which facilitate a phenotypic selection response. This technology has already allowed a major expansion in our appreciation of the potential sources of genetic variability. The myriad of control levels regulating gene expression (Darnell, 1982), identified through recombinant DNA techniques, emphasize the major role of quantitative variation in the gene product as a source of phenotypic variability. Recombinant DNA technology has also revealed that variation in gene copy number can play an important role in determining a phenotypic selection response (Schimke, 1980). Thus, one hundred and thirty years after Darwin's formal presentation of selection theory, its genetic foundations (which have been hypothesized and developed since that time) can now be directly evaluated through molecular genetic analysis of populations subjected to a specific selection pressure.

As a model for livestock species, the laboratory mouse has frequently been employed to study the phenotypic effects of specific selection criteria. Due to the importance of growth-related parameters in animal production, the imposed selection pressure has often been directed toward the enhancement/depression of a growth-related parameter (Eisen, 1975; Roberts, 1979). In mice, selection for the enhancement of body growth results in an acceleration of absolute growth rate as well as an increase in mature body size (Eisen, 1974). To better understand these changes, the indirect phenotypic responses of growth-selected mouse populations have been studied extensively. A major impetus of these studies has been to elucidate the effect of selection on the relationship between adipose and nonadipose body tissues (Fowler, 1958; Lang and Legates, 1969; McPhee and Neil, 1976). However, due to variability in the conclusions of these studies, there has been only one attempt to provide an overall interpretation of the effect of growth-selection upon adipose and nonadipose tissue

growth patterns in mice (Hayes and McCarthy, 1976).

According to the model proposed by Hayes and McCarthy (1976), 'high-growth' selected mice inevitably become fatter (relative to body weight) than unselected mice. Proponents of this model (Roberts, 1979; Allen and McCarthy, 1980) suggest that the most probable gene systems involved in a 'high-growth' selection response are those which allow an enhancement of appetite as well as those which allow an increased partitioning of energy into nonfat growth. However, since the increase of nonfat tissue eventually asymptotes, proponents further suggest that the selection-directed elevation of energy intake is then available for fat deposition; thus, according to the Hayes/McCarthy model, it is this redirection of energy into fat deposition which causes 'high-growth' selected mice to become fatter than unselected mice.

Since the proposal of this model, few reports have examined its overall validity at the phenotypic level (McPhee and Neill, 1976; Allen and McCarthy, 1980). Moreover, a classical genetic analysis of a 'high-growth' selected mouse population (Pidduck and Falconer, 1978) suggested the involvement of the growth hormone (GH) gene in the selection response; since GH has a negative effect upon carcass fat (Goodman and Grichting, 1983), this suggestion is not readily compatible with the Hayes/McCarthy model. To date, the involvement of the GH gene, in a growth-related selection response, has not been investigated at the molecular genetic level. Employing recombinant DNA techniques, in conjunction with an allometric characterization of adipose and nonadipose growth patterns, the studies described herein represent the first attempt to interpret the tissue growth patterns of 'high-growth' selected and unselected mice in terms of structural and functional variation in the GH gene. This attempt to correlate the phenotypic and molecular genetic changes in a growth-selected population permits a critical evaluation not only of the Hayes/McCarthy model, but also of the genetic foundations of selection theory itself. Moreover, these studies may also give direction to experiments which seek to enhance growth-related parameters in livestock populations via recombinant DNA technology.

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II. REDUCED RATE OF ADIPOSE TISSUE GROWTH AND LIPID ACCUMULATION IN MICE SELECTED FOR HIGH BODY WEIGHT

A. INTRODUCTION

To better understand the regulation of body growth, the indirect responses of growth-selected mouse populations have been studied extensively (for review see Eisen, 1974). A major impetus of these studies has been to elucidate the effect of selection on the relationship between fat and nonfat body tissues (Fowler, 1958; Lang and Legates, 1969; McPhee and Neill, 1976). However, genetic diversity of base populations, as well as diversity in both the selection and fat assessment criteria, has resulted in variation in the reported selection-mediated compositional changes; as a consequence, there has been only one attempt (Hayes and McCarthy, 1976) to provide an overall interpretation of the effect of growth-selection on patterns of fat and nonfat growth in mice.

According to the Hayes/McCarthy model, 'high-growth' selected mice inevitably become fatter (relative to body weight) than unselected mice (for review see Roberts, 1979). Since the proposal of this model, few reports (eg. McPhee and Neil, 1976; Allen and McCarthy, 1980) have examined its overall validity. The study reported here, therefore, investigated adipose tissue growth and lipid accumulation, within mice selected for high 42 day body weight, to provide a further critical evaluation of the Hayes/McCarthy model. Moreover, determination of the increases in both adipose tissue and extractable lipid allowed a comparison of the effectiveness of these two commonly used fat assessment criteria.

B. MATERIALS AND METHODS

Mouse Lines

The two lines of mice employed in this study were obtained from the Agriculture Canada Research Station at Lacombe, Alberta. The initial foundation population, from which

these lines were derived, was established by crossing four highly inbred strains of mice (A/J, C57-BR/cd, DBA/1, RF/J) obtained from Jackson Memorial Laboratory. The mating scheme allowed each of the four inbred lines to contribute equally to this population (Farid-Naeini, 1986). This Foundation population (FP) was maintained through 25 single pair matings per generation. Each mating produced only one litter; from each of these litters, one male and one female replacement was chosen at random. To minimize inbreeding and genetic drift, replacements were mated according to a cyclical mating plan (Farid-Naeini, 1986).

At the eighth generation of the Foundation population, a subline was established in which replacements were selected, on a within-litter basis, for high 42 day body weight. This subline (High line; HL) was maintained at a population size of 15 litters per generation (five males each mated to three females). Selection for high 42 day body weight was continued for 69 generations.

To compare rates of adipose tissue growth and lipid accumulation between growth-selected and unselected mice, representatives of the HL and FP were transferred to an environmentally controlled room in the Department of Animal Science at the University of Alberta. The room temperature was maintained at 22 ± 2°C with a relative humidity of 45 ± 5%. The room was light sealed and a controlled 12 hour light cycle was provided. The mice were housed in wire topped, polypropylene cages with deep-drawn feeders. Dried pine sawdust was used as bedding material; cages were changed once per week. The mice were fed a pelleted mouse breeder ration *ad libitum*. This ration was composed of ground whole wheat, casein, dried skim milk, dehulled 50 percent soybean meal, fish meal, corn oil, brewers yeast, stabilized Vitamin A and D, salt and ferric citrate. The manufacturer (Emory Morse Co., Box 313, Guildford, Conn., U.S.A.) guaranteed the ration to contain a minimum of 19% protein, 11% fat and 52% nitrogen free extract, and a maximum of 2.5% crude fiber.

Average 42 Day Body Weights

During the selection experiment, Agriculture Canada personnel calculated the average 42 day body weights of FP and HL mice at each generation. Weights of FP mice were not recorded between generations 33 and 63.

Dissection of Adipose Tissue Depots

The representatives of High line males (n=36), High line females (n=31), Foundation population males (n=31) and Foundation population females (n=33) were sacrificed at body weights ranging from less than 10 g to greater than 65 g. The ages of these mice ranged from 14 days to 275 days.

Dissectable adipose tissue depots were identified in five anatomical regions: the forelimb depot initiated between the shoulder blades and encircled each forelimb to end in close association with the *pectoralis superficialis*; the hindlimb depot initiated between the hindlimbs and extended cranially to cover the caudal portion of the body cavity musculature; the mesenteric depot was specifically associated with the mesenteries; the kidney depots lay between each kidney and the dorsal body wall; in males, the gonadal depot was specifically associated with the urethra and testis while, in females, this depot extended along both uterine horns and encased both ovaries.

Each of the adipose tissue depots was dissected away from the associated nonadipose tissue, individually weighed and then stored at -20°C until lipid extractions were performed. Total adipose tissue weight was computed as the sum of all adipose tissue depot weights.

To facilitate a future comparison of lipid distribution in High line and Foundation population mice, lipid extractions were performed on individual adipose tissue depots. Total lipid weight was, therefore, computed as the sum of the weight of lipid extracted from each adipose tissue depot plus the weight of lipid extracted from the nonadipose tissue (tissue remaining after the removal of the dissectable adipose tissue). The lipid extraction procedure involved a four hour ether extraction of freeze dried samples according to the A.O.A.C.

(1980) method. Technical difficulties, associated with the lipid extraction of very small adipose tissue samples, resulted in the loss of several depot lipid weights of animals sacrificed at low body weights; as a consequence, total lipid weight values were not computed for these low body weight animals.

Statistical Analysis

To represent the overall HL and FP growth patterns, body weight-age data (recorded at the time of sacrifice) were fitted to the logistic growth function,

$$Y_t = A(1-Be^{-kt})^{-1}$$

where Y = weight (g) at time t (d), B = constant of integration, e = exponent, A = asymptotic weight (g) and k = maturing constant.

Arithmetic scattergrams, of total adipose tissue weight and total lipid weight against body weight and nonadipose weight, were plotted on a within-sex basis. Each of these scattergrams exhibited an exponential trend. Therefore, all relationships between these variables were investigated using the allometric equation,

$$Y=aX^b$$

where Y = weight (g) of adipose tissue or lipid, X = weight (g) of body or nonadipose tissue, a = constant and b = relative growth coefficient. According to Berg and Butterfield (1976), b estimates "the ratio of the percentage postnatal growth of Y to the whole X".

In its logarithmic form, the allometric equation defines a linear relationship. The data were, therefore, transformed to logarithms and the linear regressions of log adipose tissue weight (and log lipid weight) on log body weight (and log nonadipose tissue weight) was computed. To better define the selection response, the HL and FP regression coefficients were compared on a within-sex basis; the statistical model for this comparison was,

$$\log_{10} Y_{ij} = A_o + L_i + B\log_{10} X_{ij} + (LB)_i\log_{10}X_{ij} + e_{ij}$$

where Y_{ij}=weight (g) of dependent variable of the ij^{th} animal, A_o=intercept, L_i=fixed effect of the i^{th} line, X_{ij}=weight (g) of independent variable of the ij^{th} animal,

B=regression coefficient of Y on X, $(LB)_j$=interaction effect (line x regression coefficient) and e_{ij}=error term (assumed to be normally and independently distributed).

C. RESULTS

Average 42 Day Body Weight Per Generation

As reflected in Figure II.1, the average 42 day body weight of FP mice remained relatively constant between generations 0 and 69 of the experiment. However, in response to the selection pressure, the average 42 day weight of HL mice increased approximately 1.5-fold during this period.

Body Weight Versus Age

A statistical comparison, of HL and FP body growth curve parameters, is presented in Chapter V. However, the logistic growth curves (Figure II.2), estimated using the weight-age data of the experimental mice, reflect the general features which were identified in Chapter V: HL mice exhibited an accelerated absolute growth rate which allowed them to achieve higher body weights, than FP mice of the same sex, at all ages. At maturity, the logistic function predicts that HL mice reached body weights which were approximately 1.5 times greater than FP mice (Figure II.2).

Total Adipose Tissue Weight Relative to Body Weight

The estimated growth coefficients (b), for total adipose tissue weight relative to body weight, are presented in Table II.1. Within each sex, the estimated HL growth coefficient is significantly lower ($P<.01$) than the FP estimate. Thus, relative to body weight, total HL adipose tissue grew at a reduced rate in comparison to that of the FP.

Scattergrams of total adipose tissue versus body weight, together with the predicted allometric pattern of adipose tissue growth, are presented in Figure II.3. These scattergrams

illustrate the reduced rate, of HL adipose tissue growth, indicated by the comparison of growth coefficients. As Figure II.3 reflects, HL and FP mice had similar adipose tissue weights at low body weights; however, as the mice increased in body weight, adipose tissue weight increased relatively more slowly in HL mice than in FP mice. As a consequence, only at fixed body weights, which exceeded the FP mature body weight, did the HL adipose tissue weight exceed that of FP mice.

Total Adipose Tissue Weight Relative to Nonadipose Tissue Weight

To remove a possible part-to-whole bias, the relationship between total adipose tissue and nonadipose tissue was also examined. The estimated growth coefficients of this relationship are presented in Table II.1. As observed for adipose tissue relative to body weight, the within-sex HL growth coefficient is lower than the corresponding FP estimate. However, the significance of the difference, between the HL and FP female coefficients (which was seen for adipose tissue relative to body weight), is reduced when adipose tissue is considered relative to nonadipose tissue. This decreased level of significance appears to be primarily a function of the larger standard errors associated with the female growth coefficients.

Arithmetic scattergrams of total adipose tissue versus nonadipose tissue, together with the predicted allometric relationship, are presented in Figure II.4. These scattergrams illustrate the very strong relationship, between adipose and nonadipose tissue, within the mouse. Also evident is a trend similar to that observed for adipose tissue weight relative to body weight: HL and FP mice had similar adipose tissue weights at low nonadipose tissue weights; however, at higher fixed nonadipose tissue weights, HL mice had less associated adipose tissue than FP mice.

Total Lipid Weight Relative to Body Weight

The growth coefficients, reflecting the rate of total lipid accumulation relative to body weight, are presented in Table II.1. Within each sex, the HL coefficient is again lower than

the FP coefficient. The scattergrams of total lipid weight versus body weight, together with the predicted allometric relationships (Figure II.5), illustrate the reduced relative rate of lipid accumulation in HL mice. While HL and FP mice had similar total lipid weights at low body weights, HL mice had lower lipid weights as body weight increased (within the range of FP body weights).

Total Lipid Weight Relative to Nonadipose Tissue Weight

Estimates of the allometric growth coefficients, for total lipid weight relative to nonadipose tissue weight, are contained in Table II.1. The general trend of reduced HL coefficients, which was established in all previous comparisons, is again evident for these two variables. The absence of significant differences, between the HL and FP estimates, results from the large associated standard errors; the magnitude of these standard errors probably reflects variation created by the extraction of very small adipose tissue samples.

This increased variation is evident in the scattergrams presented in Figure II.6. The variation does not, however, obscure the strong relationship between total lipid weight and nonadipose tissue weight. Moreover, the plots predict that, at comparable nonadipose weights, HL mice had less total lipid than FP mice.

D. DISCUSSION

The relationship, between the predicted HL and FP logistic weight-age curves, is similar to that predicted in other work (for review see Eisen, 1975). Selection of mouse populations, for the enhancement of a growth-related parameter, consistently accelerates growth rate and elevates mature body size.

Several studies, seeking to clarify the regulation of animal growth, have recognized the need to interpret this selection response in terms of changes in body tissue growth patterns (Hayes and McCarthy, 1976; McPhee and Neill, 1976; Allen and McCarthy, 1980). These studies indicated that the primary compositional change, associated with 'high-growth'

selection, was an increased rate of fat deposition relative to body weight. While their 'high' line mice were leaner than unselected or 'low' line mice at low body weights, this high relative rate of fat deposition resulted in the 'high' line mice growing fatter at higher body weights.

As indicators of fatness, the study reported here examined weight changes in both dissectable adipose tissue and ether extractable lipid; each of these variables was expressed relative to both body weight and nonadipose tissue weight. However, regardless of the manner of expression, the data yielded no indication of elevated HL adipose tissue weights, or lipid weights, throughout the range of comparable FP body and nonadipose tissue weights. In fact, Table II.1 and Figures II.3 - II.6 suggest that selection has directed a relative reduction in the rate of both adipose tissue growth, and lipid accumulation, relative to body and nonadipose tissue weight. Dissectable adipose tissue and extractable lipid, therefore, appear to be in close agreement in predicting the relative fatness of an animal.

The apparent contradiction, between previous studies and the study described here, is readily acceptable according to selection theory. A population's selection response is governed by the genetic variability present prior to the imposition of selection (together with any variation which may originate during the selection process through genetic rearrangements etc.). Therefore, genetically different 'high-growth' selection lines may achieve the characteristic acceleration of growth, and elevation of mature body size, via the involvement of different gene systems.

As suggested by the Hayes and McCarthy (1976) model, the most probable gene systems involved in a 'high-growth' selection response would be those which regulate appetite and/or energy partitioning between the major body tissues. A 'high-growth' selection line that grows at an accelerated rate, yet exhibits no change in relative tissue growth, would probably indicate that selection had acted largely upon variation at loci involved in appetite regulation. However, since their 'high' line mice exhibited an enhanced relative rate of fat deposition, McCarthy and coworkers (Hayes and McCarthy, 1976; Allen and McCarthy, 1980) suggested that, in addition to appetite-related genetic variation, selection had also acted upon genetic

variation which allowed a greater proportion of the energy intake to be partitioned into nonfat growth. As a result, the 'high' line mice were leaner at low body weights. However, since the increase of nonfat tissue eventually asymptotes during normal growth, the elevated energy intake was subsequently available for fat deposition; according to the Hayes/McCarthy model, this redirection of energy into fat deposition was responsible for the increased relative rate of fattening observed by several research groups (Hayes and McCarthy, 1976; McPhee and Neill, 1976; discussed by Roberts, 1979; Allen and McCarthy, 1980).

The data presented here suggest the need for an expansion of the basic Hayes/McCarthy model of selection-directed compositional changes. While HL mice exhibited an increase in appetite (Bailey, 1985), this increase was associated with a reduced relative rate of fat deposition. Therefore, dependent upon the genetic variation present in the selection line, 'high-growth' selection appears to be capable of coupling an increased appetite with an enhanced relative rate of nonfat tissue growth.

The specific gene systems affected by the 'high-growth' selection pressure would,thus, involve those which regulate body growth rate, mature body size and tissue growth patterns. The known metabolic influences of the growth hormone (GH) endocrine unit support the potential involvement of genes, which regulate this unit, in mediating the identified selection response: the GH endocrine unit is not only an important regulator of overall postnatal growth (Wilhelmi, 1982), but has also been demonstrated to enhance both muscle growth (Franchimont and Burger, 1975) and lipid mobilization (Goodman and Grichting, 1983). Furthermore, the GH endocrine system has been suggested to play a role in the regulation of appetite (Vaccarino et al., 1985).

Table II.1. Growth coefficients and standard errors of total adipose tissue and total lipid.

Dependent variate (y)	Independent variate (x)	Males		Females	
		High line	Foundation population	High line	Foundation population
Total Adipose Tissue	Body weight	2.413 ± .12	3.266 ± .18[a]	2.589 ± .11	3.376 ± .25[a]
	Nonadipose tissue	2.998 ± .23	4.505 ± .44[a]	3.869 ± .42	4.447 ± .56
Total Lipid	Body weight	2.821 ± .20	3.264 ± .19	2.586 ± .11	3.254 ± .28[b]
	Nonadipose tissue	3.920 ± .48	4.541 ± .45	4.219 ± .50	4.300 ± .59

[a]Within-sex growth coefficients in a row differ significantly ($P<0.01$).
[b]Within-sex growth coefficients in a row differ significantly ($P<0.05$).

Figure II.1 Response to selection for high 42 day body weight in HL mice. Selection was imposed at the eighth generation of the Foundation Population. During the selection experiment, personnel from the Agriculture Canada Research Station (Lacombe, Alberta) calculated the average 42 day body weight of HL and FP mice at each generation. Weights of FP mice were not recorded between generations 33 and 63.

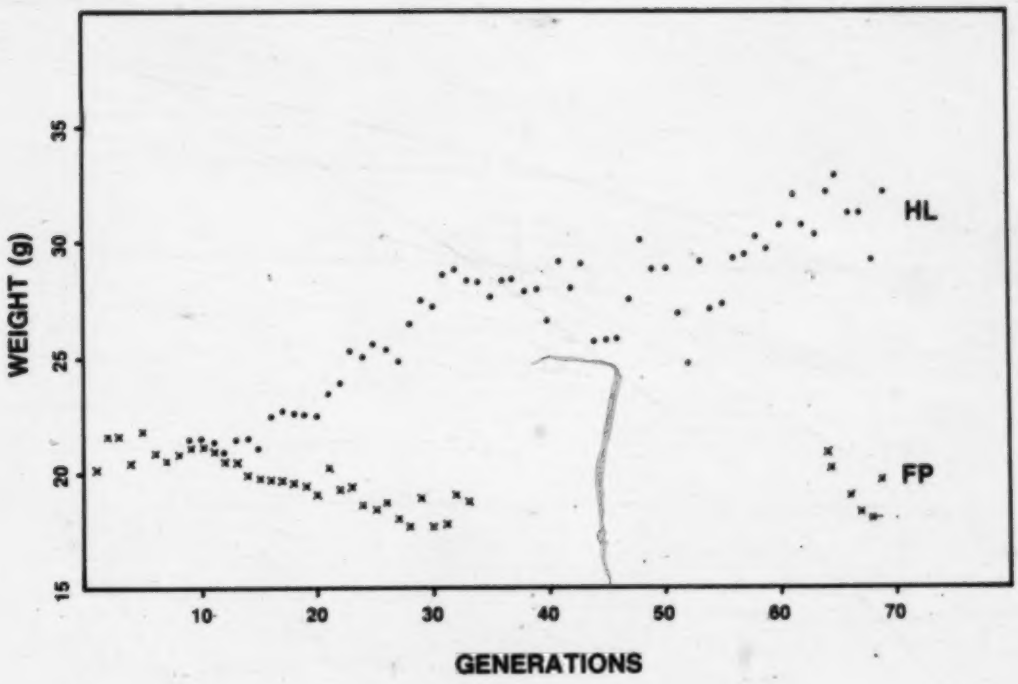
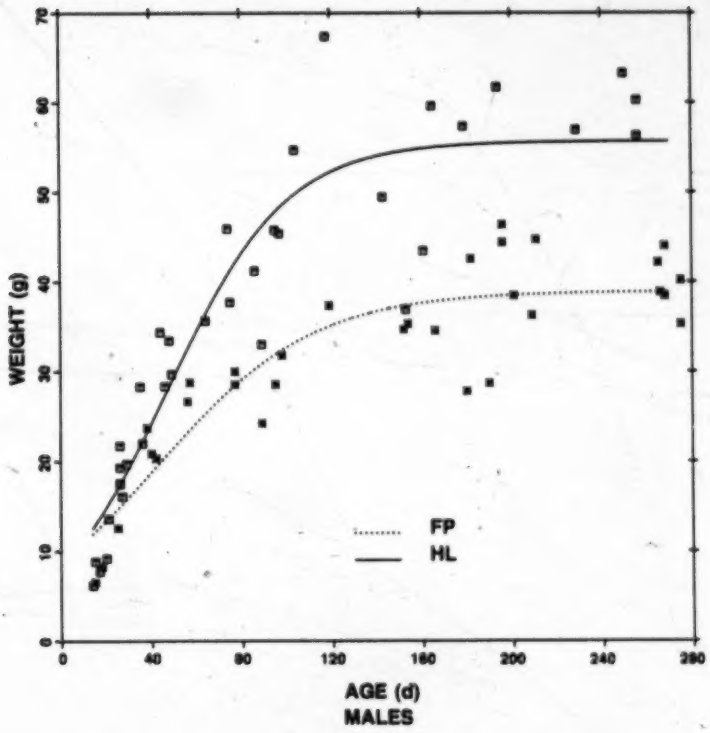
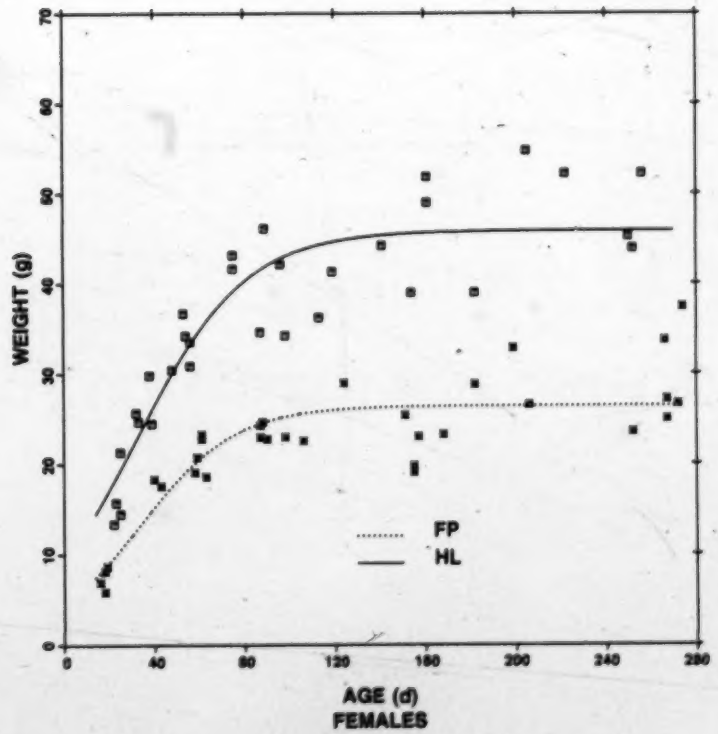

WEIGHT (g)
15
20
25
30
35
10
20
30
40
50
60
70
GENERATIONS
HL
FP

Figure II.2 Logistic weight-age curves of HL and FP mice. The representatives of HL males, HL females, FP males and FP females were sacrificed at body weights ranging from less than 10 g to greater than 65 g. Body weight-age data were recorded at the time of sacrifice. These data were then fitted to the logistic growth function which is detailed in Chapter II - Materials and Methods.

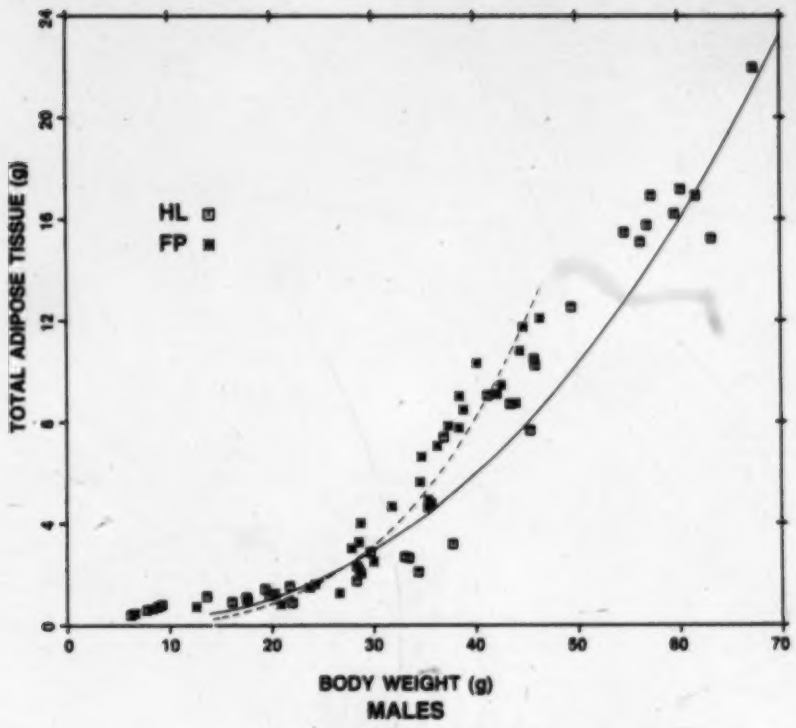


MALES



FEMALES

Figure II.3 Growth of total adipose tissue relative to body weight in HL and FP mice. The representatives of HL males, HL females, FP males and FP females were sacrificed at body weights ranging from less than 10 g to greater than 65 g. Arithmetic scattergrams of total adipose tissue versus body weight were plotted on a within-sex basis. Since each scattergram exhibited an exponential trend, the relationship between these variables was investigated using the allometric equation. The predicted allometric relationships are shown in each graph.


TOTAL ADIPOSE TISSUE (g)
HL
FP
0
4
8
12
16
20
24
0
10
20
30
40
50
60
70
BODY WEIGHT (g)
MALES

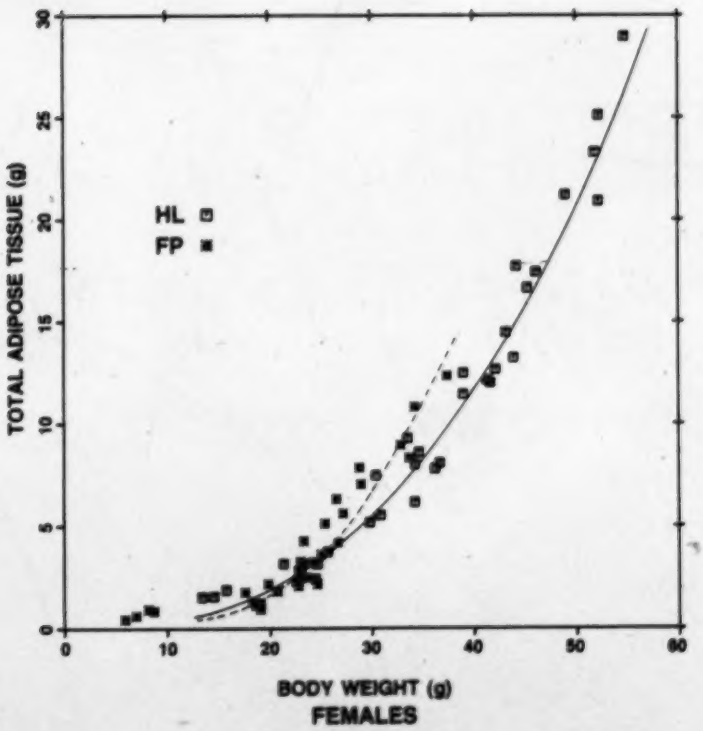
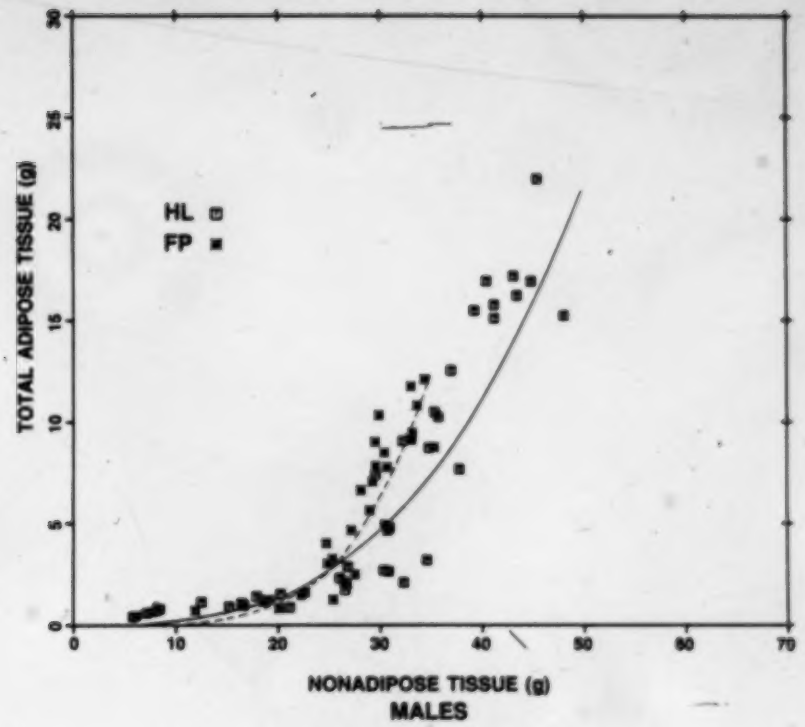

TOTAL ADIPOSE TISSUE (g)
HL
FP
0
5
10
15
20
25
30
0
10
20
30
40
50
60
BODY WEIGHT (g)
FEMALES

Figure II.4 Growth of total adipose tissue relative to nonadipose tissue in HL and FP mice. The representatives of HL males, HL females, FP males and FP females were sacrificed at body weights ranging from less than 10 g to greater than 65 g. Arithmetic scattergrams of total adipose tissue versus nonadipose tissue weight were plotted on a within-sex basis. Since each scattergram exhibited an exponential trend, the relationship between these variables was investigated using the allometric equation. The predicted allometric relationships are shown in each graph.


HL
FP
TOTAL ADIPOSE TISSUE (g)
NONADIPOSE TISSUE (g)
MALES

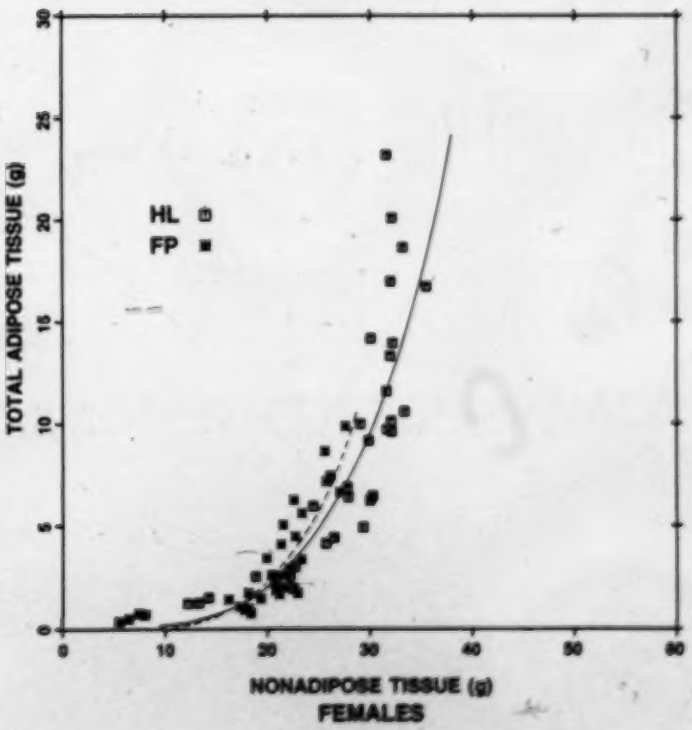
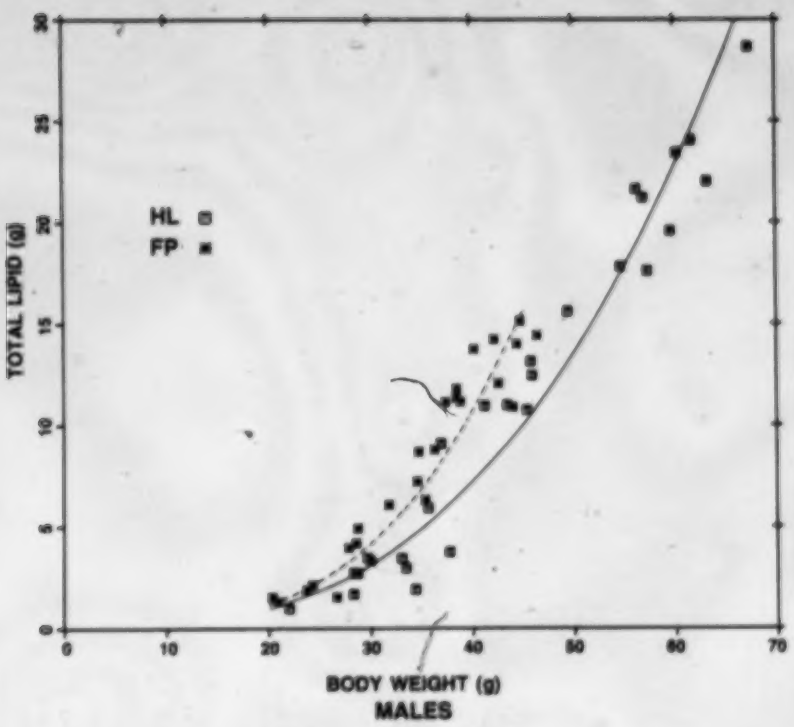

HL
FP
TOTAL ADIPOSE TISSUE (g)
NONADIPOSE TISSUE (g)
FEMALES

Figure II.5 Increase in total lipid weight relative to body weight in HL and FP mice. The representatives of HL males, HL females, FP males and FP females were sacrificed at body weights ranging from less than 10 g to greater than 65 g. Arithmetic scattergrams of total lipid weight versus body weight were plotted on a within-sex basis. Since each scattergram exhibited an exponential trend, the relationship between these variables was investigated using the allometric equation. The predicted allometric relationships are shown in each graph.


HL
FP
TOTAL LIPID (g)
BODY WEIGHT (g)
MALES

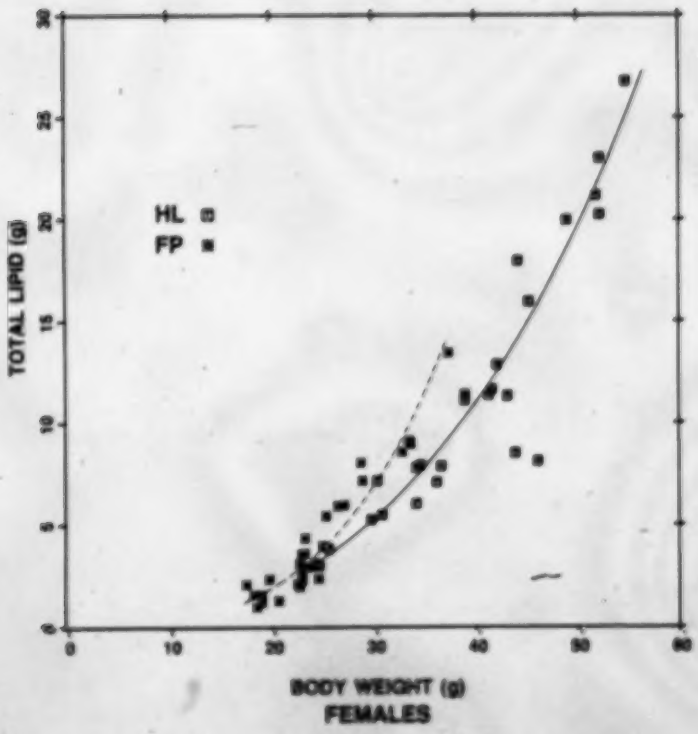
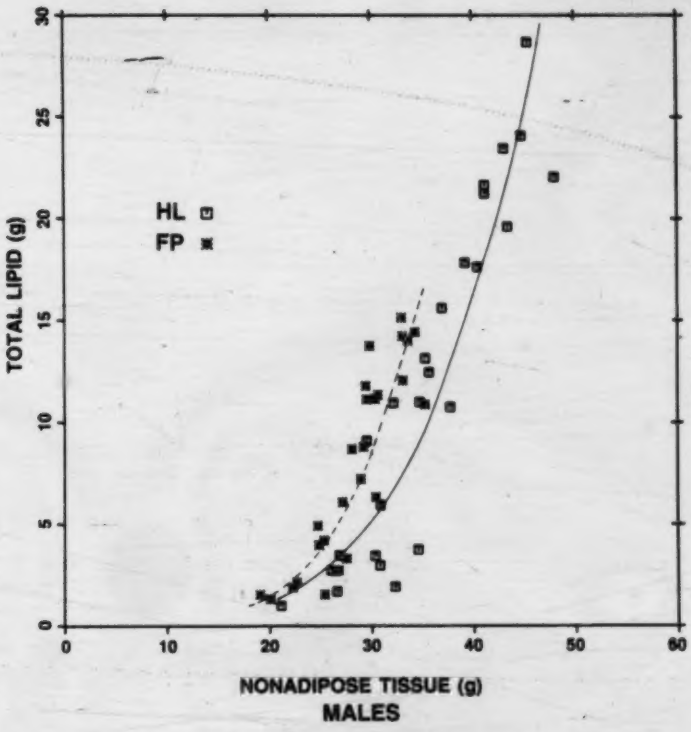
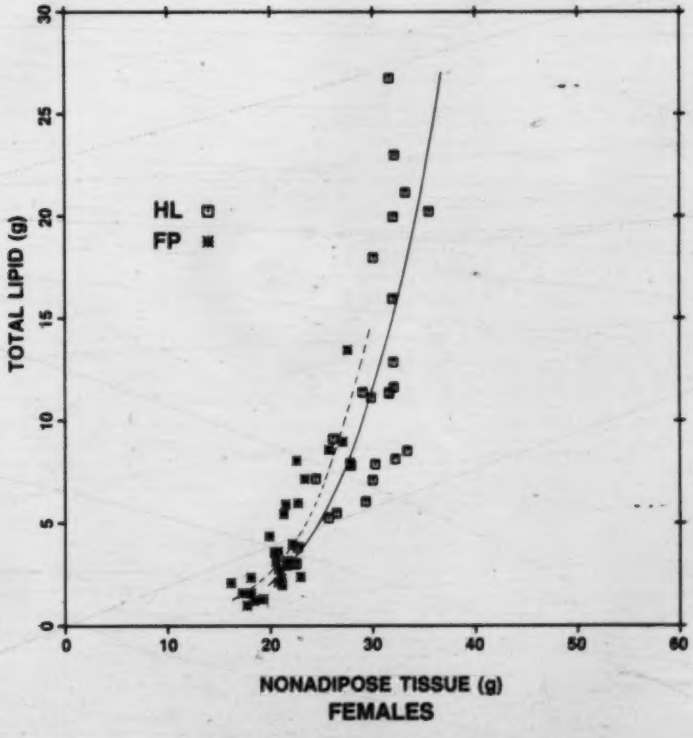

HL
FP
TOTAL LIPID (g)
BODY WEIGHT (g)
FEMALES

Figure II.6 Increase in total lipid weight relative to nonadipose tissue weight in HL and FP mice. The representatives of HL males, HL females, FP males and FP females were sacrificed at body weights ranging from less than 10 g to greater than 65 g. Arithmetic scattergrams of total lipid weight versus nonadipose tissue weight were plotted on a within-sex basis. Since each scattergram exhibited an exponential trend, the relationship between these variables was investigated using the allometric equation. The predicted allometric relationships are shown in each graph.


HL
FP
TOTAL LIPID (g)
NONADIPOSE TISSUE (g)
MALES


HL
FP
TOTAL LIPID (g)
NONADIPOSE TISSUE (g)
FEMALES

E. BIBLIOGRAPHY

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Vaccarino, F.J., Bloom, F.E., Rivier, J., Vale, W. and Koob, G.F. 1985. Stimulation of food intake in rats by centrally administered growth hormone-releasing factor. Nature. 314: 167-168.

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III. GROWTH AND BODY COMPOSITION OF MICE SELECTED FOR HIGH BODY WEIGHT

A. INTRODUCTION

The laboratory mouse has been studied extensively as a model of mammalian growth. Selection for increased body weight in mouse populations has demonstrated the feasibility of increasing growth rate and mature body weight via genetic manipulation. Since whole body growth is a biologically complex character involving the coordinated development of many different body tissues and organs, many researchers have recognized the need to interpret 'high-growth' selection responses in terms of changes in organ and tissue growth patterns (for reviews, see Eisen, 1974; Roberts, 1979; Malik, 1984). The results of these studies indicate that the accelerated growth, resulting from 'high-growth' selection, may be primarily due to an increased rate of fat accumulation; according to the interpretation proposed by Hayes and McCarthy (1976) and supported by Roberts (1979), 'high-growth' selected mice inevitably become fatter (relative to body weight) than unselected mice. However, as described in Chapter II, HL mice were leaner (relative to body weight) than unselected FP mice over the range of body weights examined. The study reported here sought to investigate the body component growth patterns of these two lines of mice, together with an additional high body weight selection line, over a range of FP weights lighter than those studied in Chapter II.

B. MATERIALS AND METHODS

Mouse Lines

The main HL and FP mouse lines employed in this study were described in Chapter II. At the eighth generation of the FP, an additional subline was derived in which replacements were selected, on a within-litter basis, for high 42 day body weight. This subline (High-Small Line; HS) was maintained at a smaller population size than the HL: 6 litters per generation (2

males each mated to 3 females). Selection for high 42 day body weight was continued for 69 generations. The HS line was maintained within the laboratory environment described in Chapter II.

Statistical Analysis

To represent the overall HL, HS and FP growth patterns, body weight-age data were fitted to the logistic growth function. This function was described in Chapter II. Arithmetic scattergrams of lipid weight, protein weight and ash weight against body weight were plotted on a within-sex basis. All relationships between these variables were investigated using the allometric equation as detailed in Chapter II. The data were, therefore, transformed to logarithms and the linear regressions of log lipid weight, log protein weight and log ash weight on log body weight were computed. To better define the selection response, the HL, HS and FP regression coefficients were compared on a within-sex basis; the statistical model for this comparison was the same as that detailed in Chapter II.

Chemical Analysis

Representatives of HL males (n=16), HL females (n=24), HS males (n=14), HS females (n=13), FP males (n=26) and FP females (n=27) were sacrificed at 21, 31, 42, 63 and 84 days of age. Individual body weights were recorded at the time of sacrifice. Following sacrifice, the mice were stored in plastic bags at -20°C.

Mouse carcasses were prepared for analysis of chemical composition according to the steam autoclaving technique outlined by Sibbald and Fortin (1981). Individual mice were placed in 600 ml beakers containing 10 ml of water; the beaker and contents were then autoclaved at 110°C for 14 h in a steam sterilizer. Sterilization and processing were achieved while maintaining 15 psi in both the jacket and the chamber. Following autoclaving, the samples were homogenized for 2 minutes in a polytron homogenizer. Homogenized samples were then frozen (-20°C) and subsequently freeze-dried for 5 days (maximum shelf

temperature of -20°C). After freeze-drying, sample weights were recorded and samples were ground to a powder.

Lipid extraction of a subsample of the ground freeze-dried material was accomplished according to the A.O.A.C. (1980) protocol. Nitrogen determinations of the lipid-free residue were accomplished using a micro-Kjeldahl auto-analyzer. Protein content was then predicted as nitrogen x 6.25. Ash was determined as the residue from subsamples, of the freeze-dried material, after ashing for 2 h at 600°C.

C. RESULTS

A statistical comparison, of HL and FP body growth curve parameters, is reported in Chapter V. The logistic growth curves (Figure III.1), estimated using the weight-age data of the experimental mice, reflect the general features which are identified in Chapter V: HL mice exhibited an accelerated absolute growth rate which allowed them to achieve higher body weights, than FP mice of the same sex, at all ages. As revealed in Figure III.1, high body weight selection had little influence on the body growth of HS mice.

The estimated growth coefficients (b), for lipid weight relative to body weight, are presented in Table III.1. Within males, the estimated HL growth coefficient was significantly lower ($P<0.05$) than the HS or FP estimate. Thus, relative to body weight, HL male lipid weight increased at a reduced rate in comparison to that of the HS or FP males. No significant differences existed among the three female lipid coefficients.

Scattergrams of lipid weight versus body weight, together with the predicted allometric pattern of lipid accumulation, are presented in Figure III.2. Figure III.2a illustrates that, while HL male lipid weights increased at a slower relative rate, HL males actually had higher absolute lipid weights (than HS or FP males) over the range of body weights examined. HL females also had higher absolute lipid weights than HS or FP females over the body weight range examined.

The estimated growth coefficients, for protein weight relative to body weight, are presented in Table III.1. As indicated, the estimated growth coefficients for the 3 mouse lines did no differ significantly within either sex.

The scattergrams of protein weight versus body weight, together with the predicted allometric relationships, are presented in Figure III.3. These scattergrams illustrate that, over the body weights examined, HL mice exhibited lower protein weights than HS or FP mice of the same body weight and sex.

The growth coefficients, reflecting the rate of ash weight increase relative to body weight, are presented in Table III.1. Within males, the growth coefficients of all three lines differed significantly in the order of HL>HS>FP. Within females, there was no significant difference in the growth coefficients for ash.

The scattergrams of ash weight versus body weight, together with their predicted allometric relationships, are presented in Figure III.4. As illustrated, over the body weights examined, HL mice exhibited lower ash weights than HS and FP mice of the same body weight and sex.

D. DISCUSSION

The relationship, between the predicted HL and FP logistic weight-age curves, was similar to that predicted in other work (for review see Eisen, 1975). Selection of mouse populations, for the enhancement of a growth-related parameter, frequently accelerates growth rate and elevates mature body size.

Several studies have sought to interpret the body compositional changes, associated with 'high-growth' selection, by examining the allometric relationships between body component weight and body weight. These studies indicated that the primary compositional change, in 'high-growth' lines, was an increased rate of fat deposition relative to body weight. While the 'high' line mice in these studies were leaner than the unselected or 'low' line mice at low body weights, the high relative rate of fat deposition caused the 'high' line mice to

grow fatter (than the unselected or 'low' line mice) as body weight increased. The age, at the imposition of selection pressure, may be a significant 'break point' in the fat development of selected mice (Fowler, 1958; Hull, 1960; Hayes and McCarthy, 1976; McPhee and Neill, 1976; Allen and McCarthy, 1980). According to these studies (discussed by Robertson, 1982 and Malik, 1985), 'high' selection mice will be leaner than unselected or 'low' mice, on a constant weight basis, prior to the age at selection; at ages following the age at selection, 'high' mice will grower fatter, relative to body weight, than unselected or 'low' mice.

Contrary to the previously cited studies, the HL mice described here exhibited higher lipid weights than FP mice at low body weights and maintained this lipid weight excess over the range of body weights examined. As indicated in Figures III.3 and III.4, these higher lipid weights were offset by lower protein and ash weights at the corresponding body weights. Also contrary to previous studies, the HL mice did not exhibit an accelerated rate of lipid accumulation relative to body weight; HL males exhibited a significantly lower lipid growth coefficient than FP males while HL and FP females showed no significant difference in their coefficients (Table III.1).

The data presented in Chapter II indicated that, at heavier body weights (from 20-70 g), HL males had a lower lipid growth coefficient than FP males. Furthermore, at body weights exceeding 25-30 g, FP male lipid weights exceeded those of HL males. Allometric analysis of female lipid weights, at FP body weights heavier than those in the present study, revealed that HL females also had a lower lipid growth coefficient than FP females. As a consequence, FP females grew fatter than HL females as body weight increased above approximately 25-30 g. Observation of the body growth curves presented here, as well as those presented in Chapter II, reveals that both HL males and females achieve body weights of 25-30 g at approximately 42 days of age (age of selection for high body weight).

The relationship between HL and FP lipid accumulation, therefore, appears to be the reverse of the relationship between other 'high' and control (or 'low') lines described in the literature. As suggested by the Hayes and McCarthy (1976) model of selection-directed

compositional changes, the most probable gene systems involved in a 'high-growth' selection response would be those which regulate appetite and/or energy partitioning between the major body tissues. A 'high-growth' selection line that grows at an accelerated rate, yet exhibits no change in relative tissue growth, would probably indicate that selection had acted largely upon variation at loci involved in appetite regulation. However, since their 'high' line mice exhibited an enhanced relative rate of fat deposition, McCarthy and coworkers (Hayes and McCarthy, 1976; Allen and McCarthy, 1980) suggested that, in addition to appetite-related genetic variation, selection had also acted upon genetic variation which allowed a greater proportion of the energy intake to be partitioned into nonfat growth. As a result, the 'high' line mice were leaner at low body weights. However, since the increase of nonfat tissue eventually asymptotes during normal growth, the elevated energy intake was subsequently available for fat deposition; according to the Hayes/McCarthy model, this redirection of energy into fat deposition was responsible for the increased relative rate of fattening observed by several research groups (Hayes and McCarthy, 1976; McPhee and Neill, 1976; discussed by Roberts, 1979; Allen and McCarthy, 1980).

The data presented here, in conjunction with that presented in Chapter II, suggest that the basic Hayes/McCarthy model requires expansion. While the HL mice described here exhibited an increased appetite (Bailey, 1985), they were fatter than FP mice only at low body weights and grew relatively leaner as body weight increased. Thus, dependent upon the genetic variation present in the selection line, 'high-growth' selection appears capable of coupling an increased appetite with a reduced relative rate of fat accumulation. That HL mice exhibited higher lipid weights, at low body weights, may indicate that their elevated appetite exceeds their genetic ability for nonfat growth during an early phase of whole body growth; however, at heavier body weights, HL mice may be better able to utilize the increased food intake for nonfat tissue growth.

While subjected to the same selection pressure as the HL line, the HS line selection response appears to have been very limited. Body growth rate and mature body size of HS

mice were similar to FP mice of the same sex. The body composition of HS and FP mice was also similar; only the male ash growth coefficient differed significantly between HS and FP mice. Thus, selection was ineffective in significantly altering the whole body growth, and compositional relationships, of HS mice relative to FP mice. While the HS selection response may reflect limited genetic variation in the founding animals, the small population size may also have played an important role. Under the cyclical mating pattern employed (Farid-Naeini, 1986), the smaller HS line population size would be expected to result in a more rapid increase in inbreeding relative to the HL line. With this increased rate of inbreeding, there would be a greater potential to restrict genetic variability, thereby compromising the ultimate genetic limits for growth-related changes in the HS line.

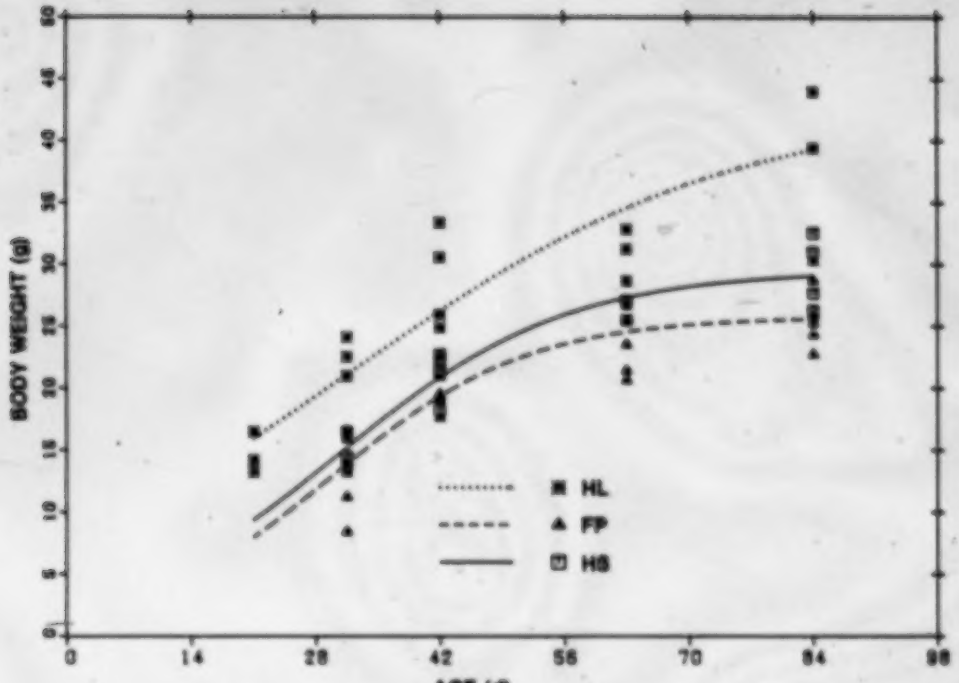
Therefore, the apparent contradiction, among the 'high-growth' selection responses reported by others and that of the two 'high' lines reported here, is compatible with selection theory. A population's selection response is governed by the genetic variability present prior to the imposition of selection (together with any variation which may originate during the selection process through genetic rearrangements, etc.). Thus, genetically different 'high-growth' selection lines may achieve the characteristic acceleration of growth, and elevation of mature body size, via the involvement of different gene systems. Furthermore, the limitation of gene pool size would be predicted to restrict a population's ability to respond to an imposed selection pressure.

Table III.1. Allometric growth coefficients and standard errors of lipid, protein and ash relative to body weight.

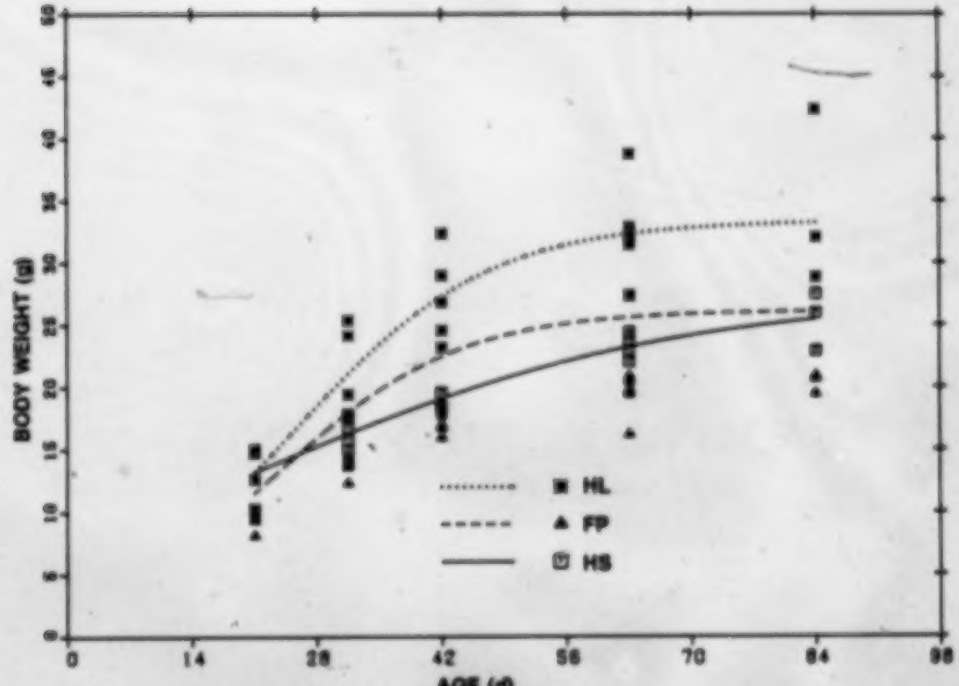
Dependent variate (Y)	Males			Females		
	HS	C	HL	HS	C	HL
Lipid	2.272 ± .32[a]	2.427 ± .19[a]	1.211 ± .21[b]	1.088 ± .36[a]	.922 ± .18[a]	1.244 ± .17[a]
Protein	.577 ± .10[a]	.596 ± .05[a]	.662 ± .10[a]	.705 ± .11[a]	.601 ± .06[a]	.614 ± .08[a]
Ash	.563 ± .06[a]	.327 ± .07[b]	.744 ± .07[c]	.954 ± .08[a]	.855 ± .05[a]	.694 ± .08[a]

[a,b,c]Within-sex coefficients (in a row) bearing different letters differ significantly ($P<0.05$).

Figure III.1 Logistic weight-age curves of HL, HS and FP mice. Representatives of each sex/line subclass were sacrificed at 21, 31, 42, 63 and 84 days of age. Individual body weights were recorded at the time of sacrifice. These data were fitted to the logistic growth function which is detailed in Chapter II - Materials and Methods.

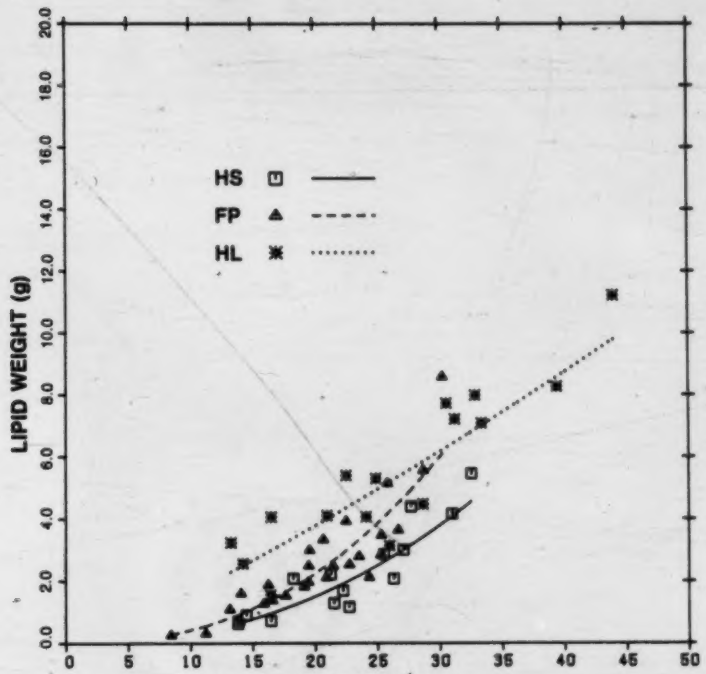


a. MALES

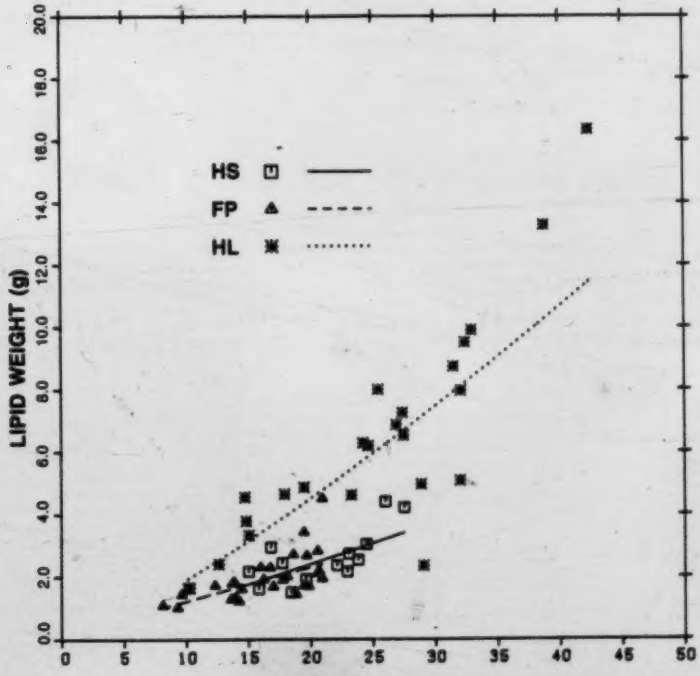


b. FEMALES

Figure III.2 Lipid increase relative to body weight in HL, HS and FP. Representatives of each sex/line subclass were sacrificed at 21, 31, 42, 63 and 84 days of age. The arithmetic scattergrams of lipid weight relative to body weight are plotted on a within-sex basis. The relationship between these variables was investigated using the allometric equation (as detailed in Chapter II). The predicted allometric relationships are plotted in each scattergram.

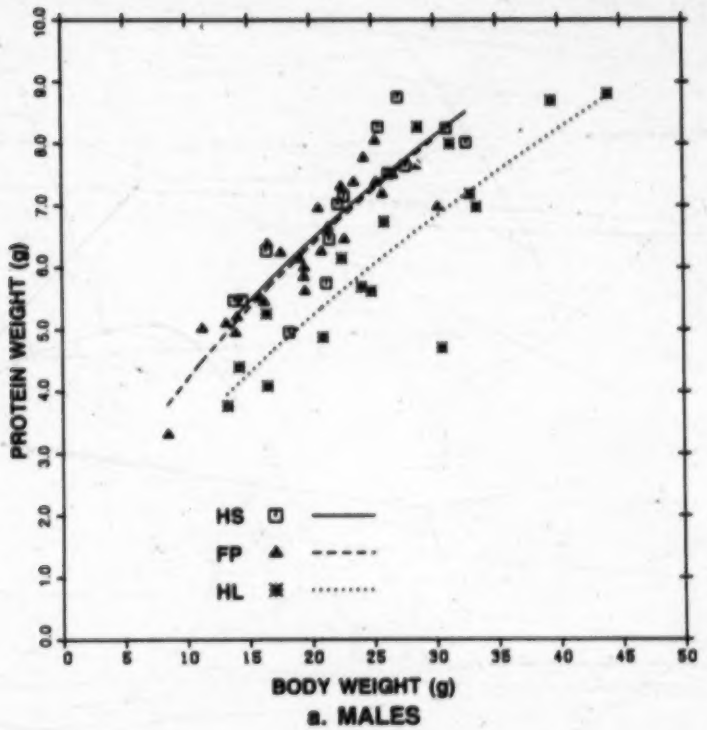


a. MALES

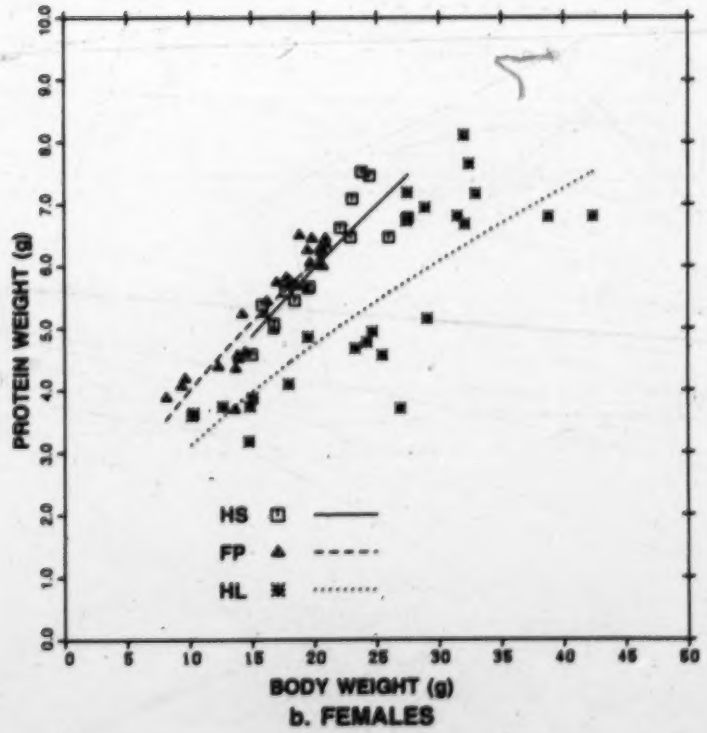


b. FEMALES

Figure III.3 Protein increase relative to body weight in HL, HS and FP mice. Representatives of each sex/line subclass were sacrificed at 21, 31, 42, 63 and 84 days of age. The arithmetic scattergrams of protein weight relative to body weight are plotted on a within-sex basis. The relationship between these variables was investigated using the allometric equation (as detailed in Chapter II). The predicted allometric relationships are plotted in each scattergram.

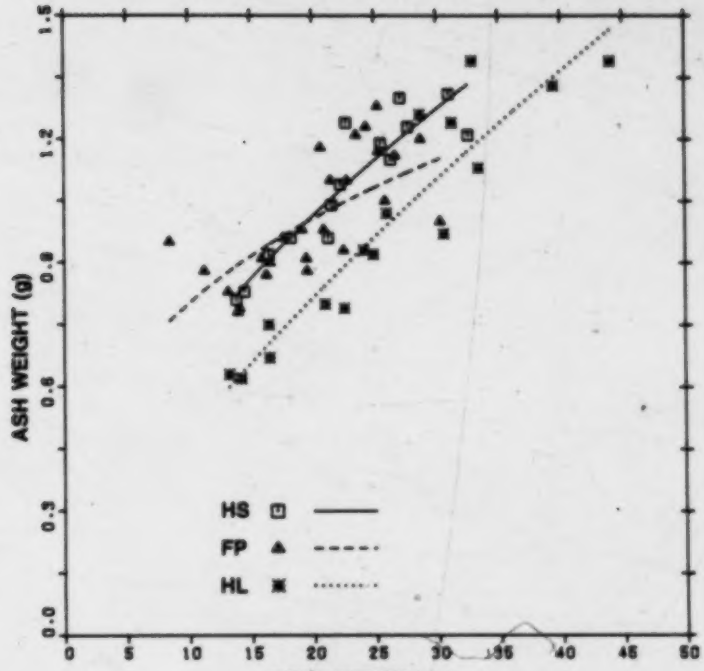


a. MALES

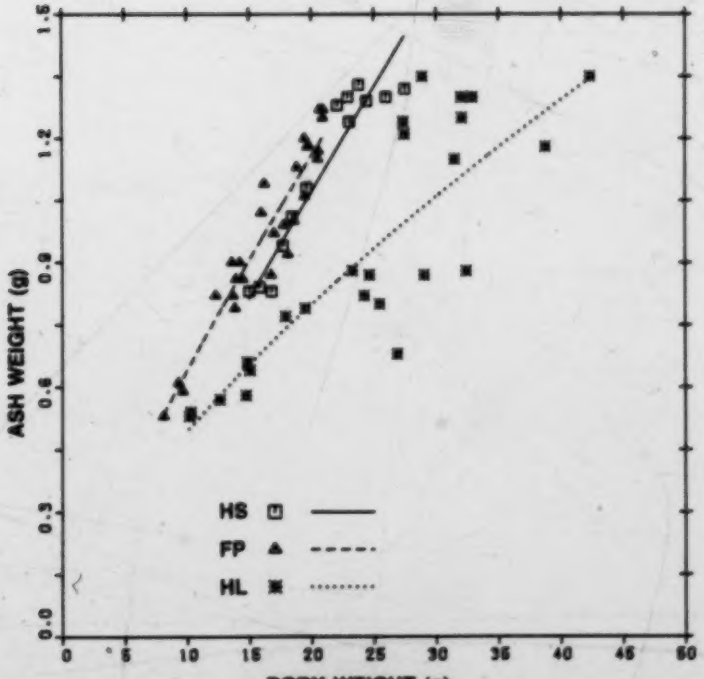


b. FEMALES

Figure III.4 Ash increase relative to body weight in HL, HS and FP mice. Representatives of each sex/line subclass were sacrificed at 21, 31, 42, 63 and 84 days of age. The arithmetic scattergrams of ash weight relative to body weight are plotted on a within-sex basis. The relationship between these variables was investigated using the allometric equation (as detailed in Chapter II). The predicted allometric relationships are plotted in each scattergram.



a. MALES



b. FEMALES

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IV. PARTITIONING AND DISTRIBUTION OF ADIPOSE TISSUE AND LIPID IN MICE SELECTED FOR HIGH BODY WEIGHT

A. INTRODUCTION

Mammalian adipose tissue plays a critical role in energy metabolism, body temperature regulation and body organ support. Within mammals, the total adipose tissue is partitioned into a number of fairly discrete adipose tissue depots in various anatomical locations. Based upon their anatomical location, these depots can be loosely regarded as either peripheral or internal depots. In many mammalian species, peripherally located depots have evolved into organs with a greater role in body temperature regulation relative to their role as sites for energy storage and mobilization (Young, 1976). Conversely, the role as an energy storage/mobilization site is relatively more important in the internal adipose tissue depots (Young, 1976).

Within both the peripheral and internal body regions, the individual adipose tissue depots appear to be distributed into specific anatomical locations which least interfere with the mammal's function. Such differences in the distribution of depots have been documented among species, breeds and sexes (Shatlock, 1909; Young, 1976; Shahin and Berg, 1985).

Therefore, while various environmental factors have been demonstrated to influence the partitioning and distribution of adipose tissue, there is clearly a strong genetic component involved in the manifestation of these two characters. To give direction to future studies of the molecular mechanisms regulating fat distribution and partitioning, the study reported here investigated the influence of 'high-growth' selection pressure on the partitioning and distribution of adipose tissue and lipid among specific peripheral and internal adipose tissue depots in mice.

B. MATERIALS AND METHODS

Mouse Lines

The history and maintenance of the HL and FP mice used in this study were described in Chapter II.

Dissection of Adipose Tissue Depots

The representatives of HL males (n=36), HL females (n=31), FP males (n=31) and FP females (n=33) were sacrificed at body weights ranging from less than 10 g to greater than 65 g. The ages of these mice ranged from 14 days to 275 days.

Dissectable adipose tissue depots were identified in five anatomical regions; the forelimb depot initiated between the shoulder blades and encircled each forelimb to end in close association with the *pectoralis superficialis*; the hindlimb depot initiated between the hindlimbs and extended cranially to cover the caudal portion of the body cavity musculture; the mesenteric depot was specifically associated with the mesenteries; the kidney depots lay between each kidney and the dorsal body wall; in males, the gonadal depot was specifically associated with the urethra and testis while, in females, this depot extended along both uterine horns and encased both ovaries. On the basis of their location, the forelimb and hindlimb depots were considered to be peripheral while the mesenteric, gonadal and kidney depots were regarded as internal depots.

Each of the adipose tissue depots was dissected away from the associated nonadipose tissue, individually weighed and then stored at -20°C until lipid extractions were performed. Total adipose tissue weight was computed as the sum of all adipose tissue depot weights.

Lipid extractions were performed on individual adipose tissue depots. Total depot lipid weight was computed as the sum of the weight of lipid extracted from each adipose tissue depot. Total body lipid was computed as the sum of the weight of lipid extracted from each adipose tissue depot plus the weight of lipid extracted from the nonadipose tissue (tissue

remaining after the removal of the dissectable adipose tissue).

The lipid extraction procedure involved a four hour ether extraction of freeze-dried samples according to the A.O.A.C. (1980) method. Technical difficulties, associated with the lipid extraction of very small adipose tissue samples, resulted in the loss of several depot lipid weights of animals sacrificed at low body weights; as a consequence, total depot and body lipid weight values, corresponding to these low body weight animals, were not computed.

Statistical Analysis

The relationships of depot adipose tissue relative to total adipose tissue, depot lipid relative to total depot lipid, and depot lipid relative to total body lipid were investigated using the allometric equation described in Chapter II. The data were, therefore, transformed to logarithms and the linear regressions of log depot adipose tissue weight on log total adipose tissue weight, and log depot lipid on log total depot lipid and log total body lipid weight, were compared. To better define the effects of selection, the HL and FP allometric regression coefficients were compared on a within-sex basis; the statistical model for this comparison was described in Chapter II. The estimated allometric parameters were then employed to adjust the dependent variable to the arithmetic mean of the independent variable. In cases where the between slopes and/or intercepts were homogenous ($P>0.05$), the common regression parameter(s) was used for adjustment.

To examine the pattern of lipid accumulation within each specific depot, linear regressions of depot lipid on depot adipose tissue were also calculated. These regressions were performed on the raw data because of the linear relationship between the two variables (Figure IV.4a-e). The statistical model employed to compare these estimated within-sex regression coefficients was,

$$Y_{ij} = A_0 + L_i + BX_{ij} + (LB)_i X_{ij} + e_{ij}$$

where Y_{ij} = weight (g) of depot lipid of the ij^{th} animal, A_0 = intercept, L_i = fixed effect of the i^{th} line, X_{ij} = weight (g) of depot adipose tissue of the ij^{th} animal, B = regression

coefficient of Y on X, $(LB)_i$ = interaction effect and e_{ij} = error term (assumed to be normally and independently distributed).

C. RESULTS

Depot Adipose Tissue Weight Relative to Total Adipose Tissue Weight

The estimated growth coefficients for the weight of the individual adipose tissue depots relative to total adipose tissue weight, as well as differences between adjusted mean depot weights, are presented in Table IV.1. Comparison of the depot coefficients (within each line/sex subclass) indicates that the relative growth of the five depots can be ranked generally as forelimb < mesenteric, hindlimb < gonadal, kidney.

Arithmetic scattergrams of each depot versus total adipose tissue, together with the predicted allometric relationships, are presented in Figure IV.1a-e and Figure IV.2a-e. Within males, Figure IV.1b,c,d reveals that the selection pressure had little influence upon the relative growth patterns of the forelimb, mesenteric and gonadal adipose tissue depots. However, as seen in Figure IV.1a, HL and FP hindlimb adipose tissue depots did not exhibit the same pattern of increase relative to total adipose tissue; as a result, at the total adipose tissue arithmetic mean, the HL adjusted hindlimb depot weight was 23.1% less than the FP adjusted mean (Table IV.1). As shown in Figure IV.1e and Table IV.1, the pattern of kidney adipose tissue growth also differed between HL and FP males. The higher relative growth of HL kidney adipose tissue dictated that HL males had 18.3% more kidney adipose tissue at the arithmetic mean of total adipose tissue (Table IV.1). Moreover, at the maximum FP total adipose tissue weight (11.62 g), HL males had 51.2% more kidney adipose tissue.

Within females, the HL and FP hindlimb, forelimb and mesenteric depots growth coefficients differ significantly ($P<0.05$) (Table IV.1). However, the biological significance of the differences, in the relative growth of these depots, is not clear. Figure IV.2b,c suggest that, while HL females may have exhibited higher forelimb and mesenteric adipose tissue

weights at very low body weights, differences between the lines were less clear at higher comparable body weights. The growth patterns of kidney and gonadal adipose tissue were very similar between HL and FP females.

Depot Lipid Weight Relative to Total Depot Lipid Weight

The estimated growth coefficients for individual depot lipid weights relative to total depot lipid weights, as well as the differences between the adjusted mean depot lipid weights, are presented in Table IV.2. Comparison of the depot coefficients (within each line/sex subclass) indicates that the relative rate of depot lipid accumulation can be ranked generally as follows: forelimb < hindlimb, mesenteric < gonadal, kidney. Only HL males coefficients deviate from this general ranking; within this subclass, gonadal lipid exhibited a lower relative rate of increase than hindlimb lipid.

Arithmetic scattergrams of depot lipid relative to total depot lipid, together with the predicted allometric relationships, are shown in Figure IV.3a-e and Figure IV.4a-e. Similar to the trend observed for depot adipose tissue relative to total adipose tissue, HL males appeared to partition more lipid into the kidney depot, and less into the hindlimb depot, than FP males. While the HL and FP mesenteric and gonadal growth coefficients differ significantly, observation of Figure IV.3b,c,d indicates that the pattern of accumulation of mesenteric, gonadal and forelimb lipid (relative to total depot lipid) is similar in the males of both lines.

Within females, observation of Table IV.2 and Figure IV.4a,c,d,e suggests that the accumulation of hindlimb, mesenteric, gonadal and kidney lipid (relative to total depot lipid) was very similar between the HL and FP mice. HL and FP females did, however, appear to differ in their pattern of forelimb lipid accumulation: HL females had higher forelimb lipid weights at the low total lipid weights, yet exhibited a lower forelimb growth coefficient. As a result, HL and FP females had similar forelimb lipid weights at heavier total lipid weights (Figure IV.4b).

Depot Lipid Weight Relative to Total Body Lipid Weight

Table IV.3 contains the estimated growth coefficients for weight of individual depot lipid, relative to total body lipid weight, as well as the mean depot lipid weights adjusted to the within-sex arithmetic mean of total body lipid weight. Within FP mice, the relative rate of increase in depot lipid can be ranked as nonadipose < forelimb < hindlimb <mesenteric < gonadal < kidney. Within HL mice, the nonadipose lipid also exhibited the lowest relative rate of increase; however, the ranking of the other depots not only differed between the sexes, but also deviated from the FP ranking.

Arithmetic scattergrams of depot lipid relative to total body lipid, together with the predicted allometric relationships, are presented in Figure IV.5a-f and Figure IV.6a-f. While all of the estimated depot regression coefficients differ between HL and FP males, the scattergrams and the differences between adjusted depot means suggest that HL and FP males had similar weights of forelimb, mesenteric, gonadal and nonadipose lipid over the entire range of common total body lipid weights. However, following the trend identified in the two previous comparisons, HL males appeared to partition a lower proportion of their total body lipid into the hindlimb depot; they compensated for this by partitioning proportionately more of their total body lipid into the kidney depot than FP males.

In females, the relative accumulation patterns of hindlimb, mesenteric, gonadal and kidney depot lipid were very similar between the two lines. Moreover, while the nonadipose lipid growth coefficients differed significantly, females of both lines had similar nonadipose lipid weights over the entire range of common total body lipid weights. As in the previous comparisons between HL and FP females, FP females had less forelimb lipid at low total body lipid weights. However, the lower HL forelimb regression coefficient allowed FP females to achieve forelimb lipid weights, similar to those of HL females, at heavier total lipid weights (Figure IV.6b).

Depot Lipid Weight Relative to Depot Adipose Tissue Weight

Arithmetric scattergrams of depot lipid versus depot adipose tissue, as well as the predicted linear relationships between these variables, are presented in Figure IV.7a-e and Figure IV.8a-e. The regression coefficients, which describe the slopes of these relationships, are presented in Table IV.4. The magnitude of these coefficients reflects the high degree of saturation ($\geq$ 80% lipid w/w) of all depots over the range of adipose tissue weights examined. Table IV.4 indicates the existence of several significant within-sex line differences in the rate of depot lipid accumulation. However, as illustrated in the corresponding figures, these differences were very small.

D. DISCUSSION

Variation in the partitioning and distribution of fat has been well documented among numerous species. Since the anatomical location of fat can influence both form and function, this variation undoubtedly originated as each species evolved toward a maximal level of fitness within a specific environment. In many species, peripheral depots play a relatively more important role (than internal depots) in body temperature regulation, while internal fat depots play a relatively greater role in energy storage and mobilization (Young, 1976). At the biochemical level, this 'division of labor' between internal and peripheral depots is strongly associated with their metabolic activities; the more easily mobilized internal depots exhibit a higher rate of fatty acid turnover than the peripheral depots (Schultz and Ferguson, 1974).

Within the peripheral and internal regions of the body, the individual adipose tissue depots appear to be distributed in specific anatomical locations which least interfere with an animal's function. Marine mammals have adapted to their cold environments by distributing a thick layer of subcutaneous fat over their entire body. However, while of great adaptive value to marine mammals, such a distribution of subcutaneous fat would be a barrier to heat dissipation for animals inhabiting warmer environments. Desert mammals (e.g. camel, fat-tailed and fat-rumped sheep, fat-tailed marsupial mouse) which need to accumulate fat as

an energy source during times of food shortage, yet cannot allow subcutaneous fat to interfere with heat loss, restrict peripheral fat depots to highly localized body regions to reduce their impact upon temperature regulation (Young, 1976). The localization of peripheral fat, into the buttocks region of desert mammals, has also been described in certain African tribes (Shatlock, 1909).

Differences in fat partitioning have also been characterized among breeds of livestock. As in natural populations, the breed-specific patterns of fat partitioning can be related to variation in selection pressures. Dairy cattle, which must maintain a readily mobilizable energy source to meet heavy lactational demands, tend to partition a higher proportion of their fat into the internal depots (Butler-Hogg and Wood, 1982); alternatively, the traditional British beef breeds, which have been selected for a smooth blocky conformation, tend to have more fat in the subcutaneous depots (Charles and Johnson, 1976).

Many studies of mouse populations, which have been selected for the enhancement of a growth-related parameter, have reported that this selection pressure accelerates the rate of total fat accumulation relative to body weight increase (Hayes and McCarthy, 1976; Allen and McCarthy, 1980; reviewed by Malik, 1984). Moreover, Allen and McCarthy (1980) revealed that individual fat depots were capable of responding differentially to this increase in fat accumulation; in the range of common body weights, their 'high-growth' selection lines had more forelimb and mesenteric fat, but less gonadal and kidney fat, than their 'low-growth' lines. This change in fat distribution and partitioning may reflect changes necessary to accommodate changes in the form or function of the 'high-growth' mice.

Contrary to the other reported 'high-growth' selection lines, the HL mice described here exhibited a reduced rate of total fat growth (relative to body weight) in comparison to the 'non-growth' selected FP mice (Chapter II). As reported here, this depression of the relative rate of fat accumulation had little influence upon the developmental relationships among the various depots. However, in comparison with FP males, HL males appeared to partition more fat into the kidney depot, and less into the hindlimb depot, over the range of

common total fat weights. This shift in partitioning, from an internal to a peripheral depot, is almost the reverse of the changes observed in the 'high-growth' line of Allen and McCarthy (1980). Since the Allen and McCarthy line exhibited an enhanced rate of total fat accumulation relative to body weight, changes in the rate of total fat accumulation may therefore be associated with changes in the internal versus peripheral partitioning of fat.

Within females, with the exception of minor differences in the relative growth of forelimb fat, no obvious line differences existed in either the partitioning or the distribution of fat.

The potential of a population to alter the developmental relationships among depots, in response to 'high-growth' selection, is governed by the genetic variability present prior to the imposition of selection (together with any variation which may originate during the selection process through genetic rearrangements, etc.). The limited change in fat partition/distribution, associated with the dramatic acceleration of HL body growth (Chapters II, III and V), may reflect the existence of little genetic variability for fat partitioning in the founding animals. Alternatively, the increased growth rate and mature body size of the HL mice may have necessitated very little alteration in fat partition/distribution to accommodate any functional changes associated with the acceleration of growth.

Table IV.1. Growth coefficients and standard errors of depot adipose tissue relative to total adipose tissue.

Depot	Male		% difference in adjusted means[1]	Females		% difference in adjusted means[1]
	High	Foundation		High	Foundation	
Hindlimb	1.074 ± .02	1.127 ± .03	23.1	1.004 ± .02	0.932 ± .02[a]	
Forelimb	0.780 ± .02	0.715 ± .04	0.0	0.631 ± .02	0.736 ± .03[b]	12.5
Mesenteric	0.917 ± .05	0.999 ± .07	0.0	0.721 ± .03	1.122 ± .04[b]	14.7
Gonadal	1.435 ± .05	1.321 ± .06	0.0	1.377 ± .06	1.251 ± .04	19.7
Kidney	1.548 ± .04	1.177 ± .07[b]	18.3	1.392 ± .03	1.370 ± .05	0.0

[a]Within-sex coefficients in a row differ significantly ($P<.05$).
[b]Within-sex coefficients in a row differ significantly ($P<.01$).
[1]Adjusted to arithmetic mean of total adipose tissue (males = 6.01 g and females = 5.67 g).

Table IV.2. Growth coefficients and standard errors of depot lipid relative to total depot lipid.

Depot	Male		% difference in adjusted means[1]	Females		% difference in adjusted means[1]
	High	Foundation		High	Foundation	
Hindlimb	1.109 ± .02	0.994 ± .02	40.7	0.914 ± .05	0.939 ± .03[b]	0.0
Forelimb	0.895 ± .03	0.954 ± .03[b]	0.0	0.671 ± .05	0.879 ± .05[b]	19.0
Mesenteric	0.924 ± .03	1.101 ± .05[b]	17.5	0.898 ± .07	1.076 ± .04	8.7
Gonadal	0.873 ± .04	1.015 ± .03[a]	19.6	1.188 ± .03	1.093 ± .04	8.2
Kidney	1.183 ± .02	1.098 ± .04	44.5	1.219 ± .07	1.223 ± .05	0.0

[a]Within-sex coefficients in a row differ significantly ($P<.05$).
[b]Within-sex coefficients in a row differ significantly ($P<.01$).
[1]Adjusted to arithmetic mean of total depot lipid (males = 5.70 g and females = 4.73 g).

Table IV.3. Growth coefficients and standard errors of depot lipid relative to total body lipid.

Depot	Male		% difference in adjusted means[1]	Females		% difference in adjusted means[1]
	High	Foundation		High	Foundation	
Hindlimb	1.249 ± .05	1.134 ± .02[a]	29.1	1.129 ± .04	1.207 ± .05[b]	0.0
Forelimb	0.976 ± .03	1.115 ± .05[a]	39.2	0.835 ± .04	1.142 ± .06[b]	12.9
Mesenteric	1.066 ± .03	1.336 ± .07[b]	9.2	1.233 ± .09	1.372 ± .08	0.0
Gonadal	0.998 ± .07	1.184 ± .04[a]	6.5	1.451 ± .05	1.394 ± .08	0.0
Kidney	1.297 ± .03	0.929 ± .08[b]	61.1	1.679 ± .06	1.593 ± .07	0.0
Nonadipose	0.902 ± .03	0.815 ± 0.2[b]	5.5	0.666 ± 0.3	0.749 ± .02[a]	15.8

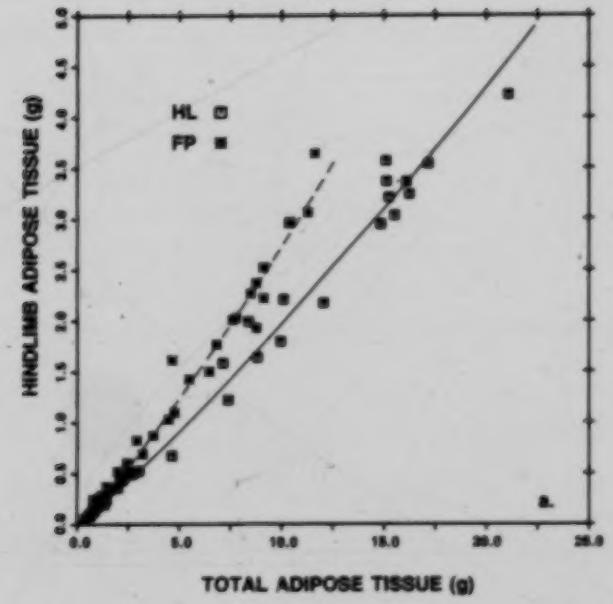
[a]Within-sex coefficients in a row differ significantly ($P<.05$).
[b]Within-sex coefficients in a row differ significantly ($P<.01$).
[1]Adjusted to arithmetic mean of total body lipid (males = 10.65 g and females = 7.86 g).

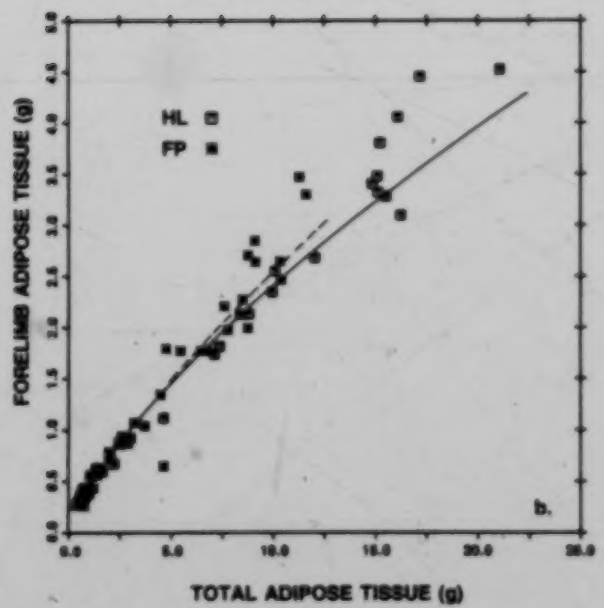
Table IV.4 Growth coefficients for depot lipid relative to depot adipose tissue.

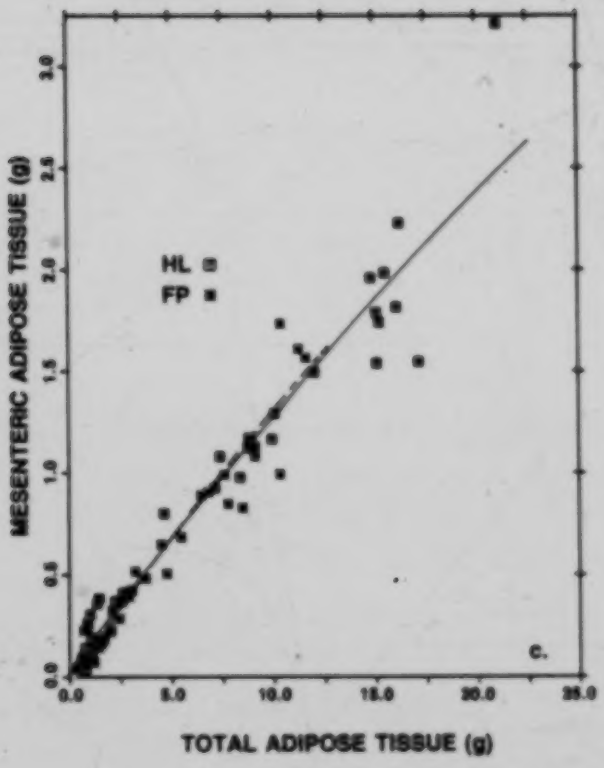
	Male		Females	
Depot	High	Foundation	High	Foundation
Hindlimb	0.918 ± .02	0.889 ± .02	0.934 ± .02	0.893 ± .01[b]
Forelimb	0.822 ± .02	0.809 ± .01	0.834 ± .02	0.793 ± .02[b]
Mesenteric	0.902 ± .01		0.922 ± .03	0.819 ± .02[a]
Gonadal	0.845 ± .01	0.893 ± .02[a]	0.929 ± .00	0.926 ± .02
Kidney	0.847 ± .00	0.793 ± .02[b]	0.874 ± .01	0.788 ± .01[b]

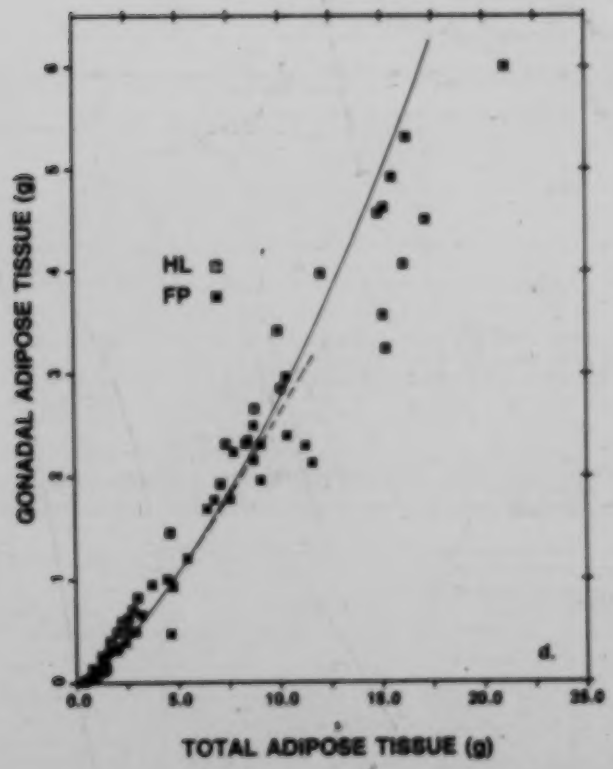
[a]Within-sex coefficients in a row differ significantly ($P<.05$).
[b]Within-sex coefficients in a row differ significantly ($P<.01$).

Figure IV.1(a-e) Depot adipose tissue weight relative to total adipose tissue weight in HL and FP mice (Males). The relationship, between the adipose tissue of a specific depot and total adipose tissue, was investigated using the allometric equation described in Chapter II. The predicted allometric relationship between these variables is plotted in each scattergram.


HL
FP
HINDLIMB ADIPOSE TISSUE (g)
TOTAL ADIPOSE TISSUE (g)
a.


HL
FP
FORELIMB ADIPOSE TISSUE (g)
TOTAL ADIPOSE TISSUE (g)
b.


MESENTERIC ADIPOSE TISSUE (g)
HL
FP
c.
TOTAL ADIPOSE TISSUE (g)


GONADAL ADIPOSE TISSUE (g)
HL
FP
d.
TOTAL ADIPOSE TISSUE (g)

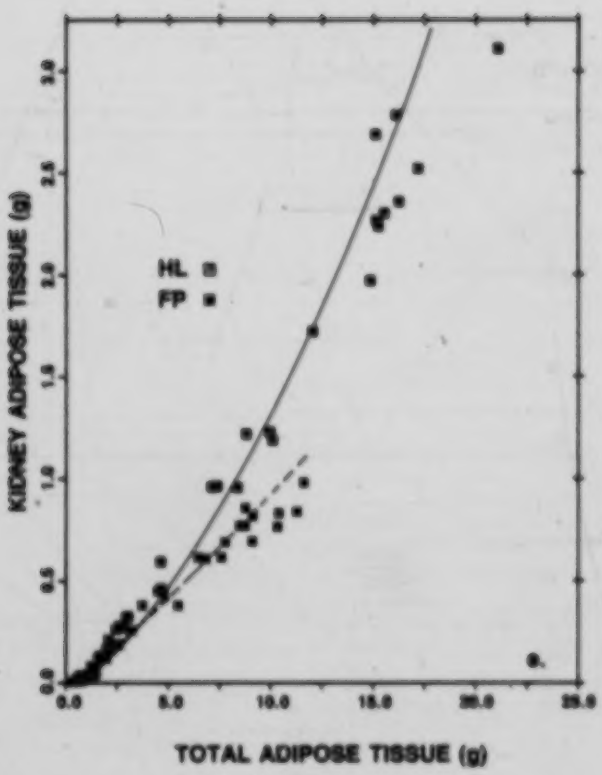
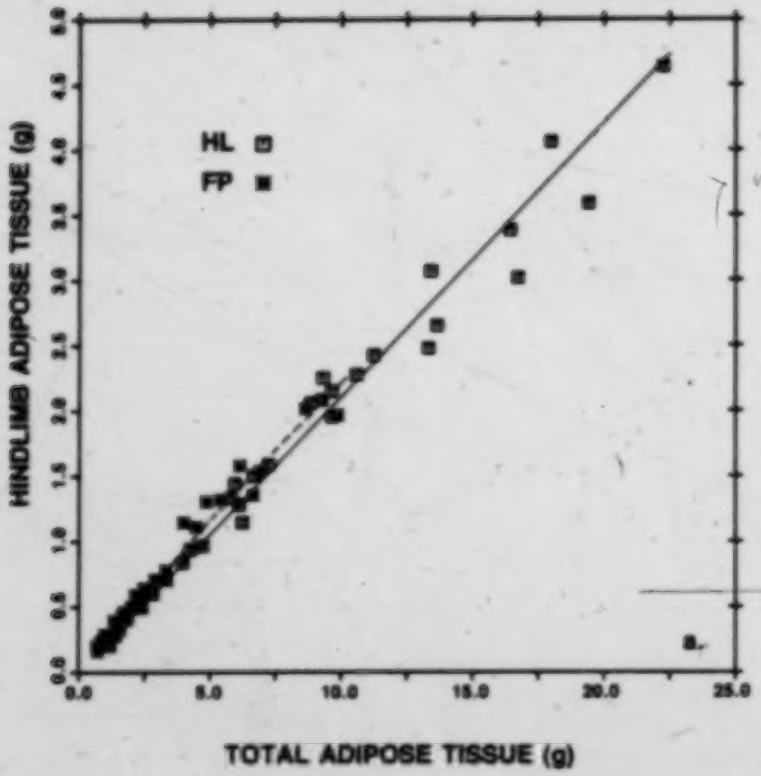
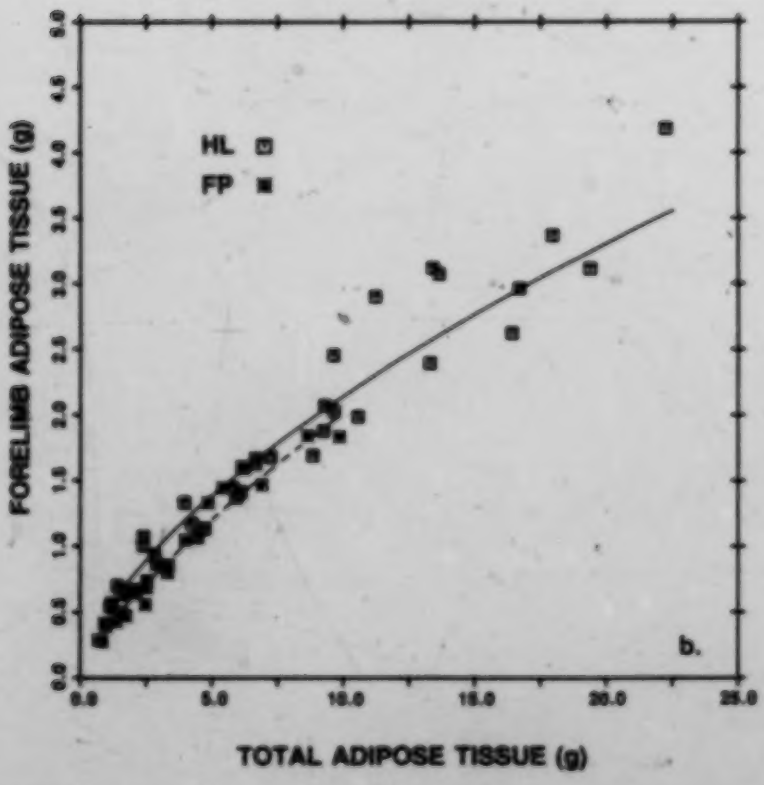
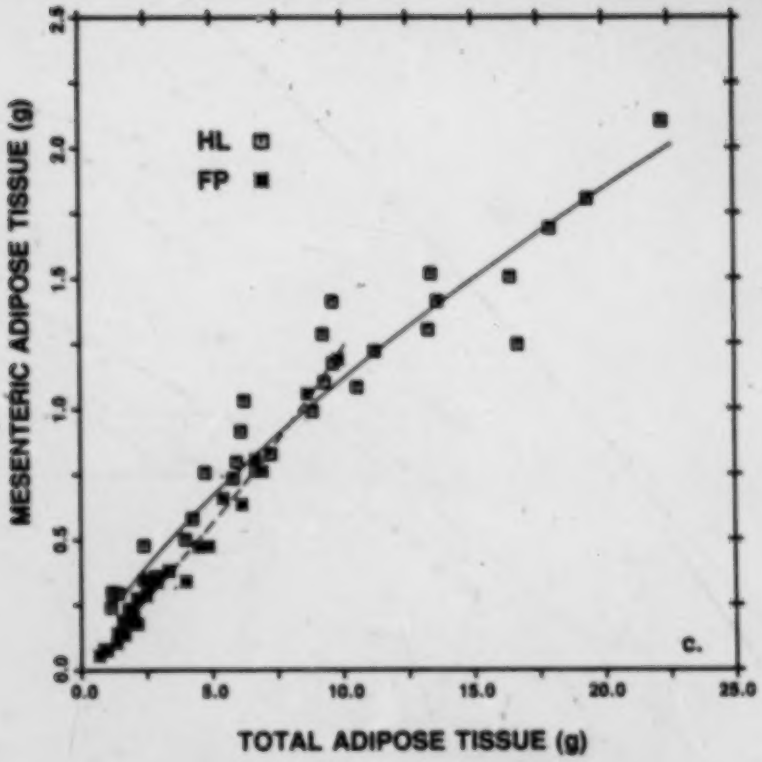
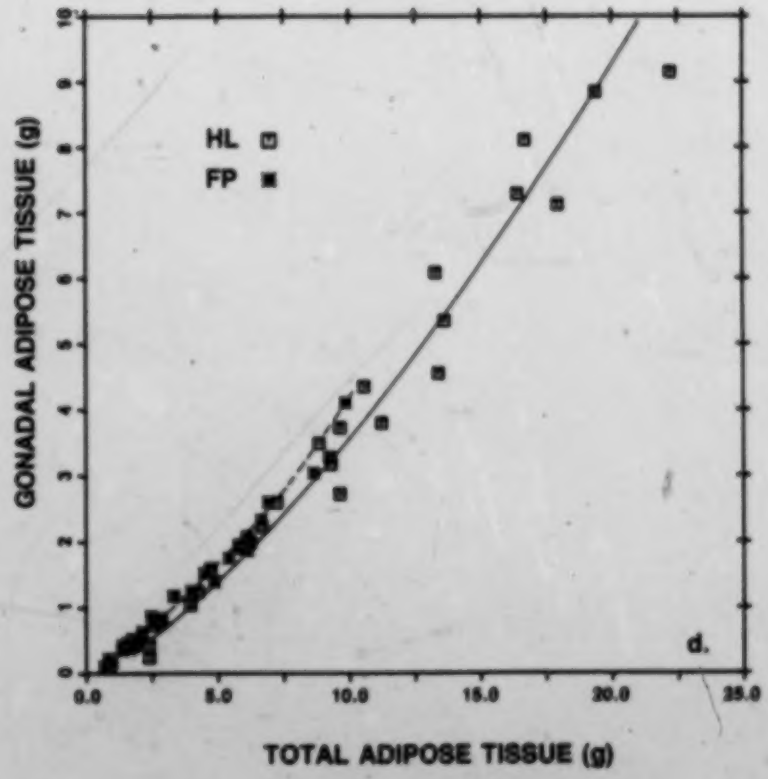

KIDNEY ADIPOSE TISSUE (g)
TOTAL ADIPOSE TISSUE (g)
HL
FP
0.0
0.5
1.0
1.5
2.0
2.5
3.0
5.0
10.0
15.0
20.0
25.0

Figure IV.2(a-e) Depot adipose tissue weight relative to total adipose tissue weight in HL and FP mice (Females). The relationship, between the adipose tissue of a specific depot and total adipose tissue, was investigated using the allometric equation described in Chapter II. The predicted allometric relationship between these variables is plotted in each scattergram.


HL
FP
HINDLIMB ADIPOSE TISSUE (g)
TOTAL ADIPOSE TISSUE (g)
a.


HL
FP
FORELIMB ADIPOSE TISSUE (g)
TOTAL ADIPOSE TISSUE (g)
b.


MESENTERIC ADIPOSE TISSUE (g)
HL
FP
c.
0.0
5.0
10.0
15.0
20.0
25.0
TOTAL ADIPOSE TISSUE (g)


GONADAL ADIPOSE TISSUE (g)
HL
FP
d.
0.0
5.0
10.0
15.0
20.0
25.0
TOTAL ADIPOSE TISSUE (g)

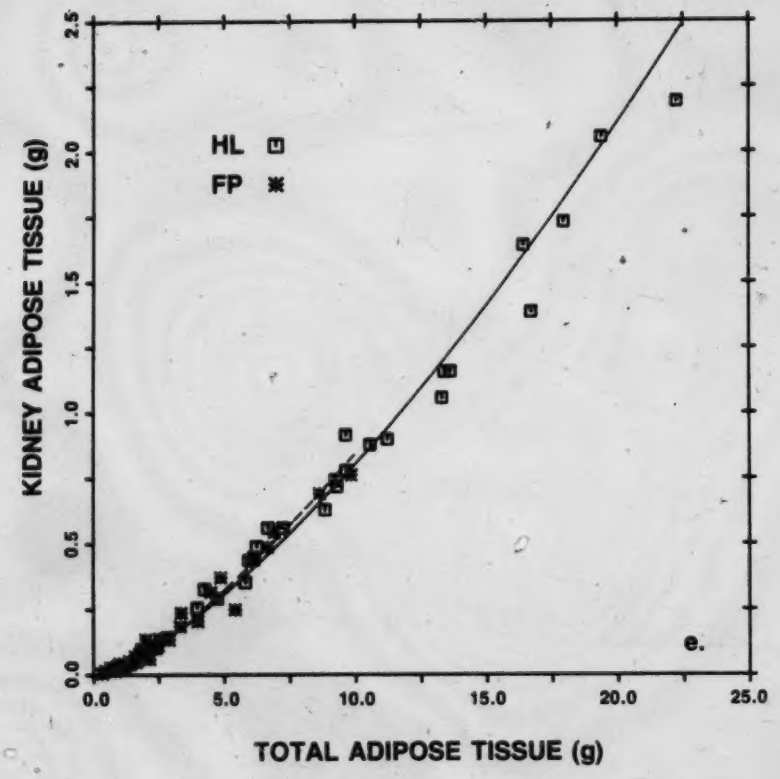
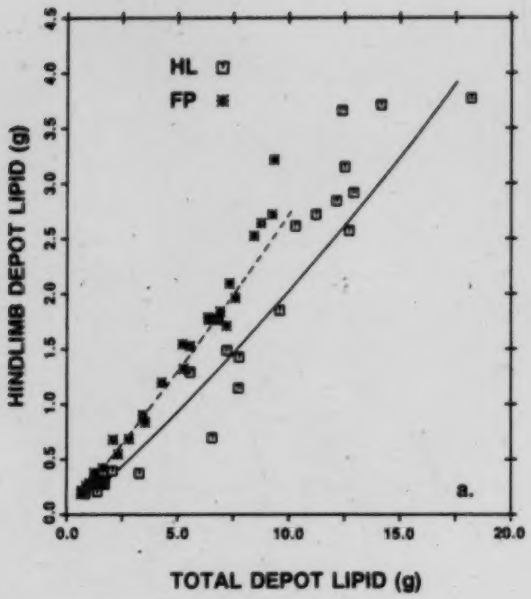
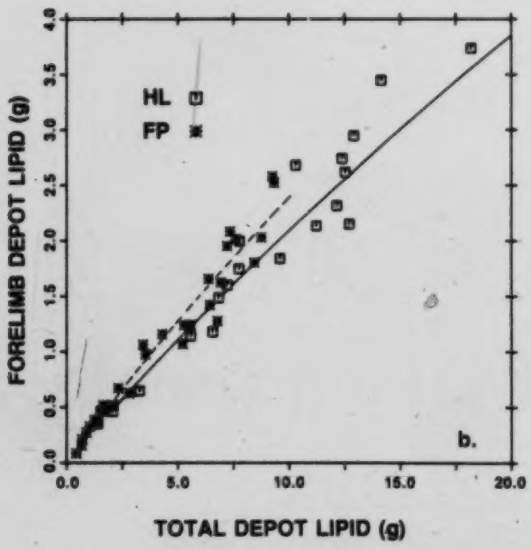
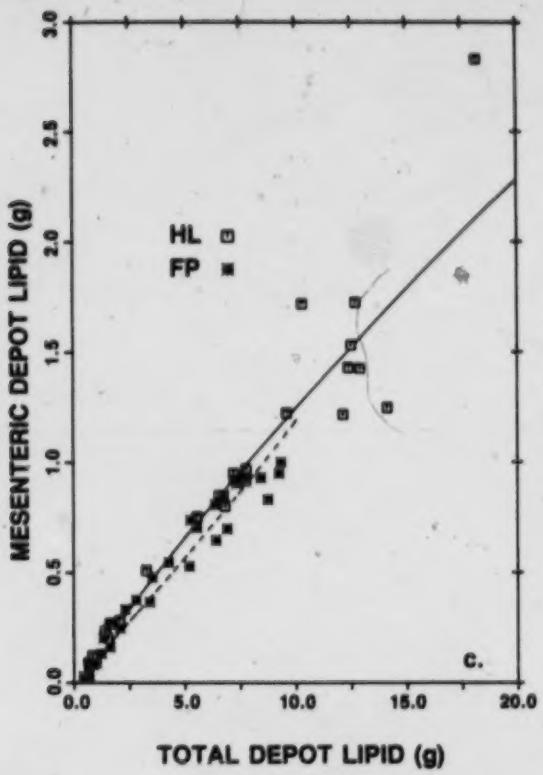
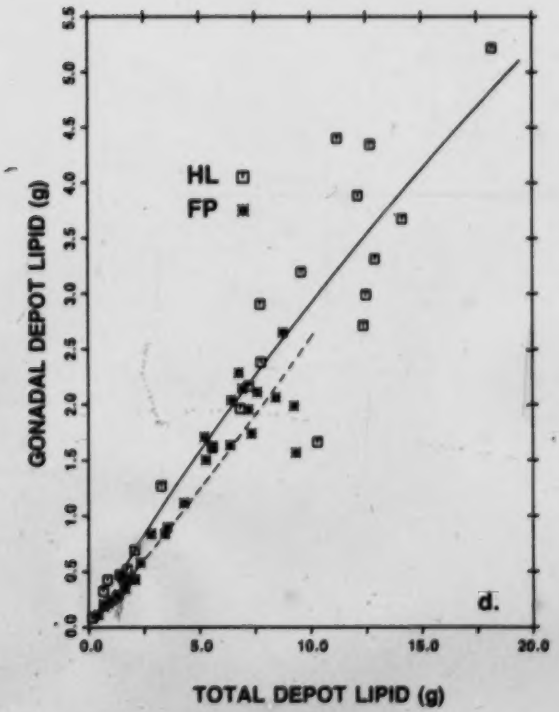

KIDNEY ADIPOSE TISSUE (g)
TOTAL ADIPOSE TISSUE (g)
HL
FP
e.
0.0
0.5
1.0
1.5
2.0
2.5
0.0
5.0
10.0
15.0
20.0
25.0

Figure IV.3(a-e) Depot lipid weight relative to total depot lipid weight in HL and FP mice (Males). The relationship, between the lipid weight of a specific depot and total depot lipid weight, was investigated using the allometric equation described in Chapter II. The predicted allometric relationship between these variables is plotted in each scattergram.


HL
FP
HINDLIMB DEPOT LIPID (g)
TOTAL DEPOT LIPID (g)
a.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
10.0
15.0
20.0


HL
FP
FORELIMB DEPOT LIPID (g)
TOTAL DEPOT LIPID (g)
b.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
5.0
10.0
15.0
20.0


MESENTERIC DEPOT LIPID (g)
HL
FP
c.
TOTAL DEPOT LIPID (g)


GONADAL DEPOT LIPID (g)
HL
FP
d.
TOTAL DEPOT LIPID (g)

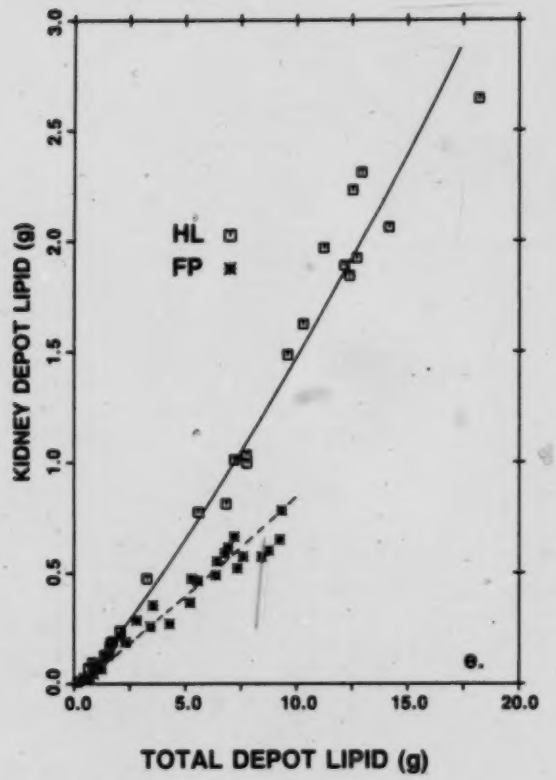
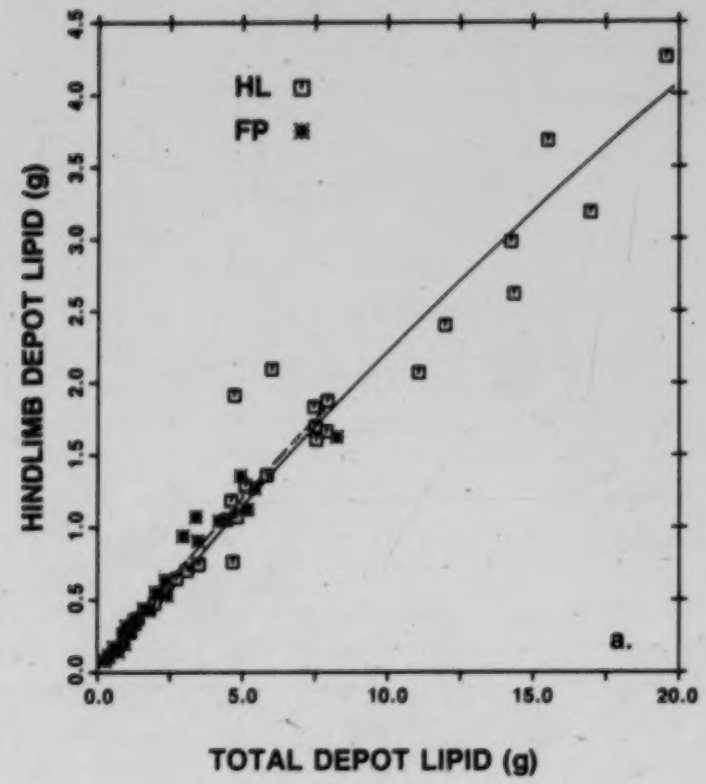
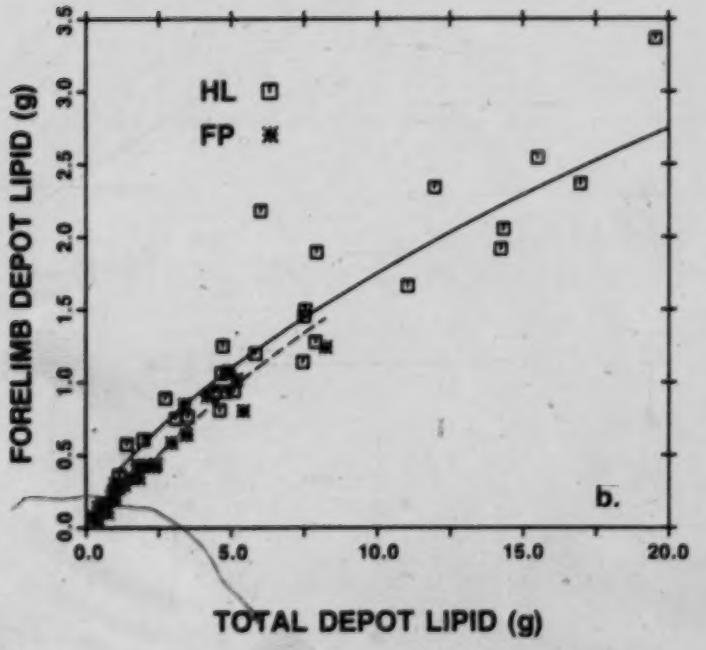
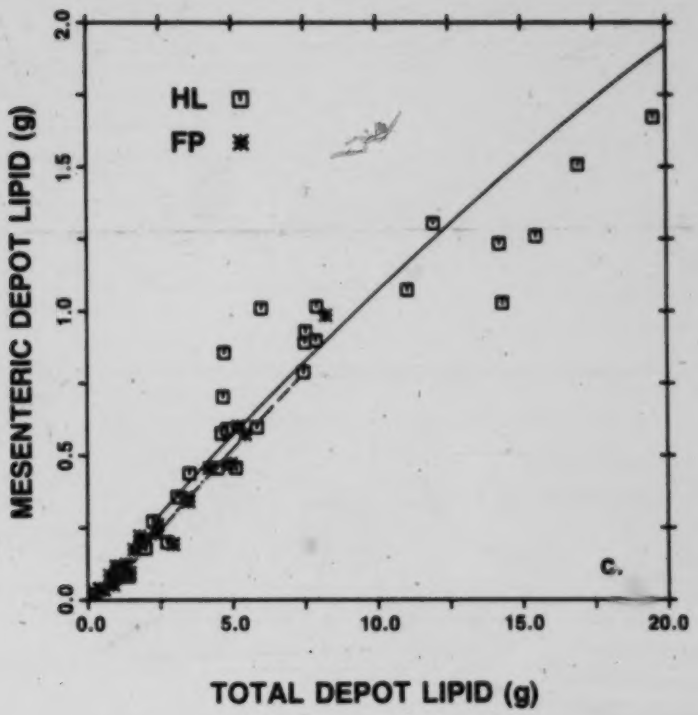
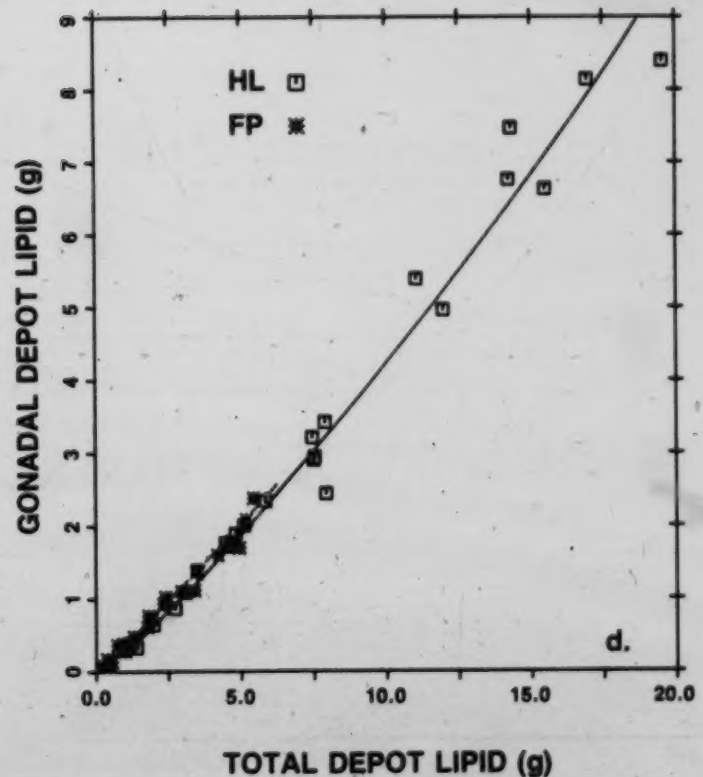

KIDNEY DEPOT LIPID (g)
TOTAL DEPOT LIPID (g)
HL
FP
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.0
5.0
10.0
15.0
20.0

Figure IV.4(a-e) Depot lipid weight relative to total depot lipid weight in HL and FP mice (Females). The relationship, between the lipid weight of a specific depot and total depot lipid weight, was investigated using the allometric equation described in Chapter II. The predicted allometric relationship between these variables is plotted in each scattergram.


HL
FP
HINDLIMB DEPOT LIPID (g)
TOTAL DEPOT LIPID (g)
a.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
10.0
15.0
20.0


HL
FP
FORELIMB DEPOT LIPID (g)
TOTAL DEPOT LIPID (g)
b.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
5.0
10.0
15.0
20.0


MESENTERIC DEPOT LIPID (g)
HL
FP
c.
0.0
0.5
1.0
1.5
2.0
0.0
5.0
10.0
15.0
20.0
TOTAL DEPOT LIPID (g)


GONADAL DEPOT LIPID (g)
HL
FP
d.
0
1
2
3
4
5
6
7
8
9
0.0
5.0
10.0
15.0
20.0
TOTAL DEPOT LIPID (g)

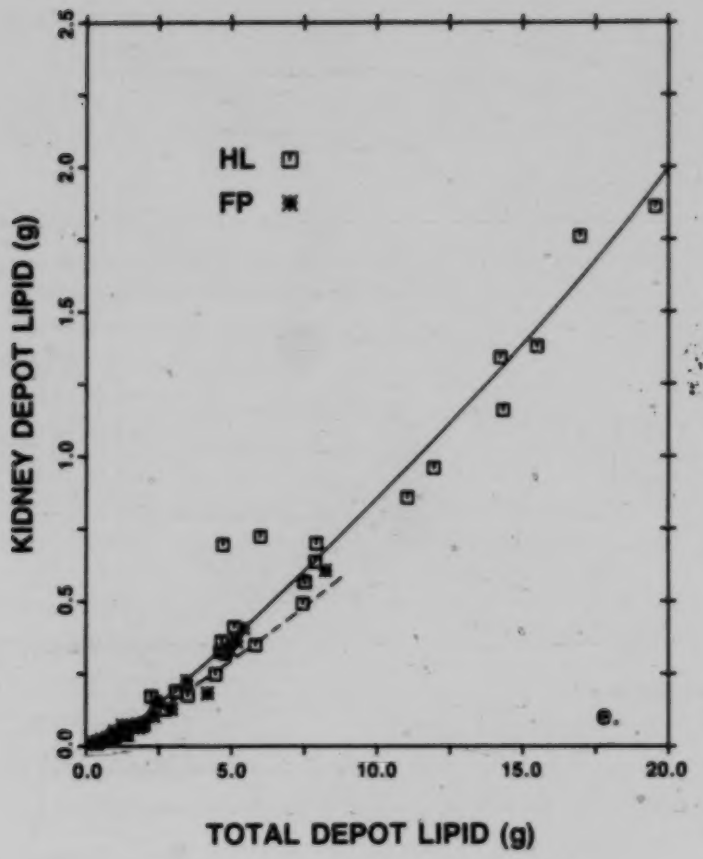
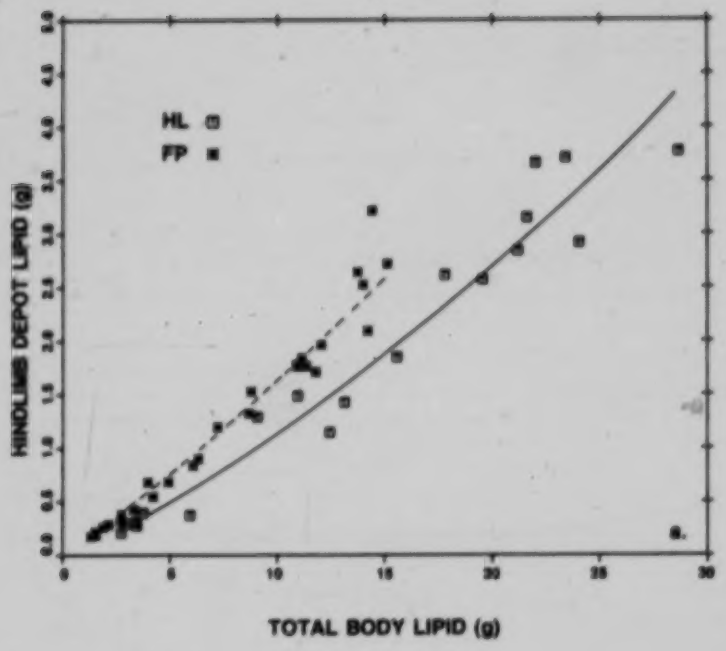
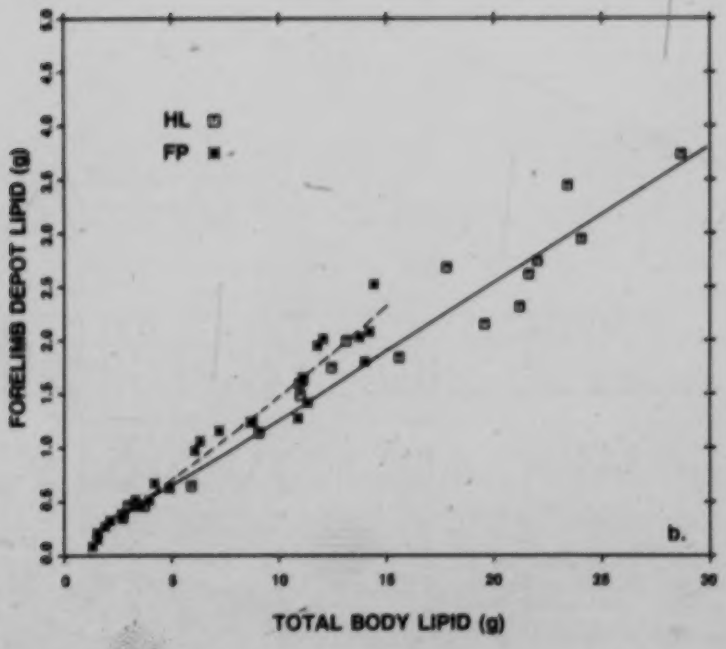
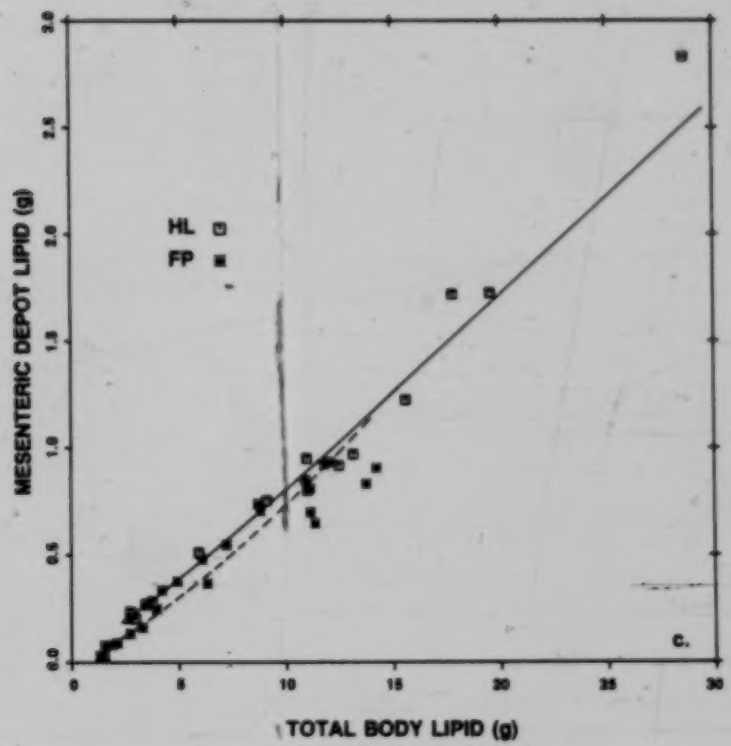
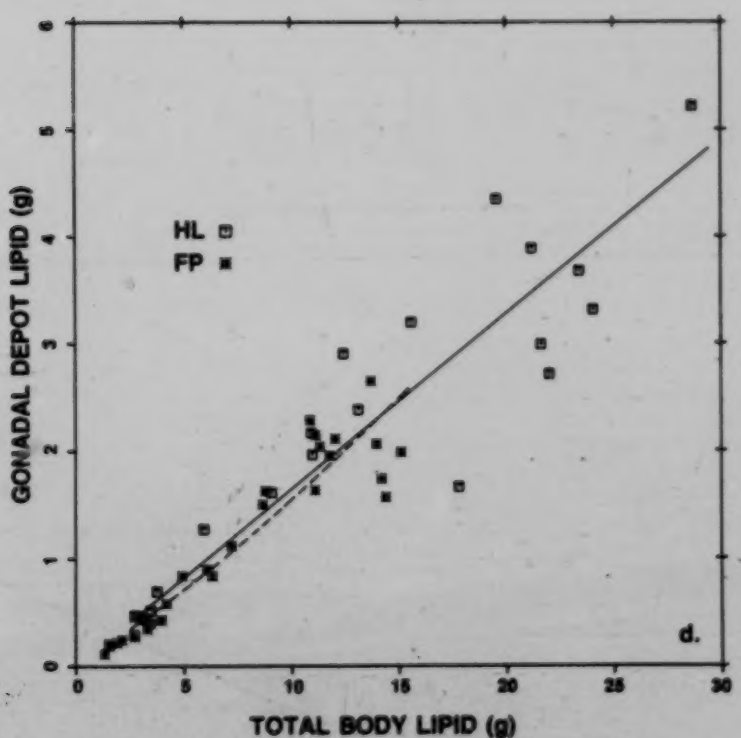

HL
FP
KIDNEY DEPOT LIPID (g)
TOTAL DEPOT LIPID (g)
0.0
0.5
1.0
1.5
2.0
2.5
0.0
5.0
10.0
15.0
20.0

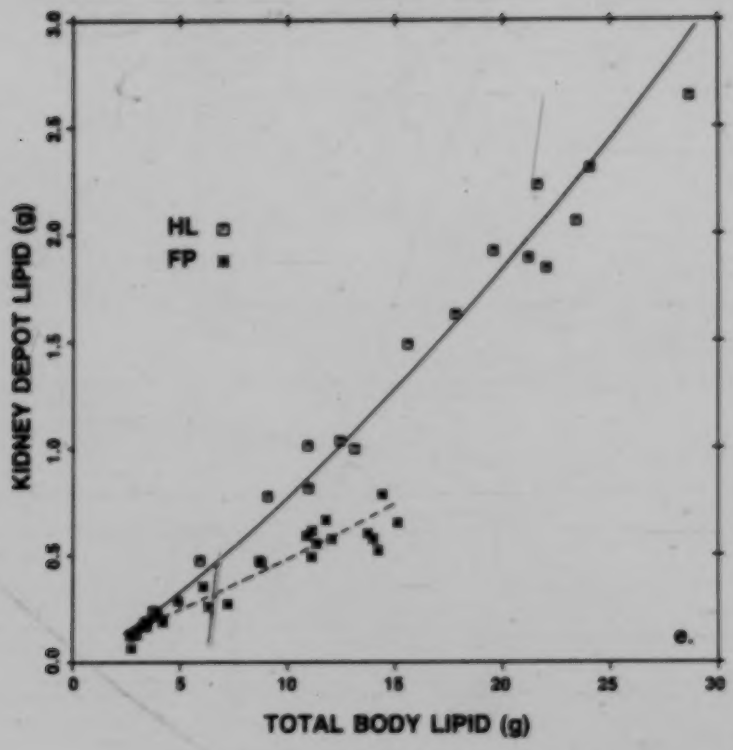
Figure IV.5(a-f) Depot lipid weight relative to total body lipid weight in HL and FP mice (Males). The relationship, between the lipid weight of a specific depot and total body lipid weight, was investigated using the allometric equation described in Chapter II. The predicted allometric relationship between these variables is plotted in each scattergram.


HL
FP
HINDLIMB DEPOT LIPID (g)
TOTAL BODY LIPID (g)
a.


HL
FP
FORELIMB DEPOT LIPID (g)
TOTAL BODY LIPID (g)
b.


MESENTERIC DEPOT LIPID (g)
HL
FP
c.
TOTAL BODY LIPID (g)


GONADAL DEPOT LIPID (g)
HL
FP
d.
TOTAL BODY LIPID (g)


KIDNEY DEPOT LIPID (g)
HL
FP
e.
TOTAL BODY LIPID (g)

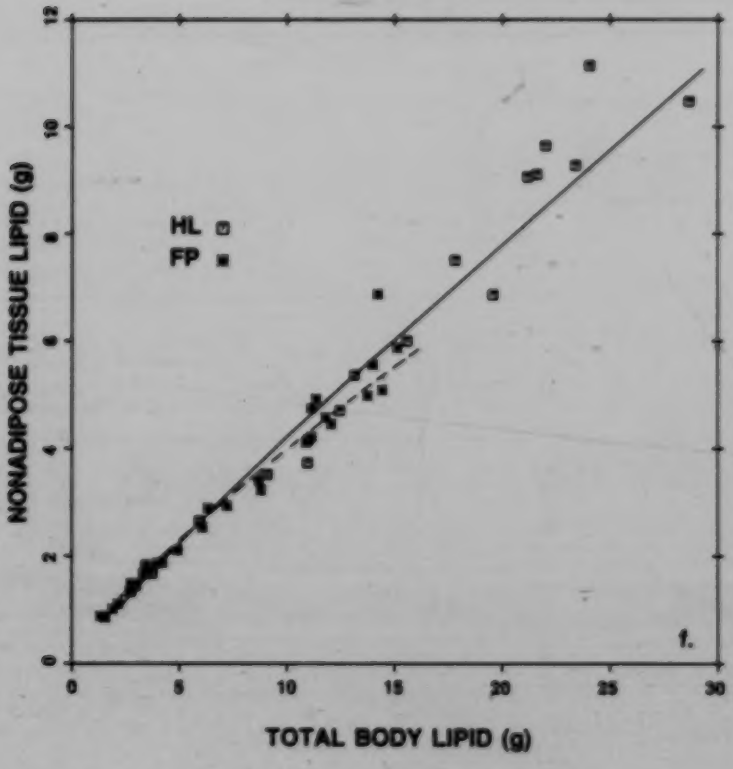
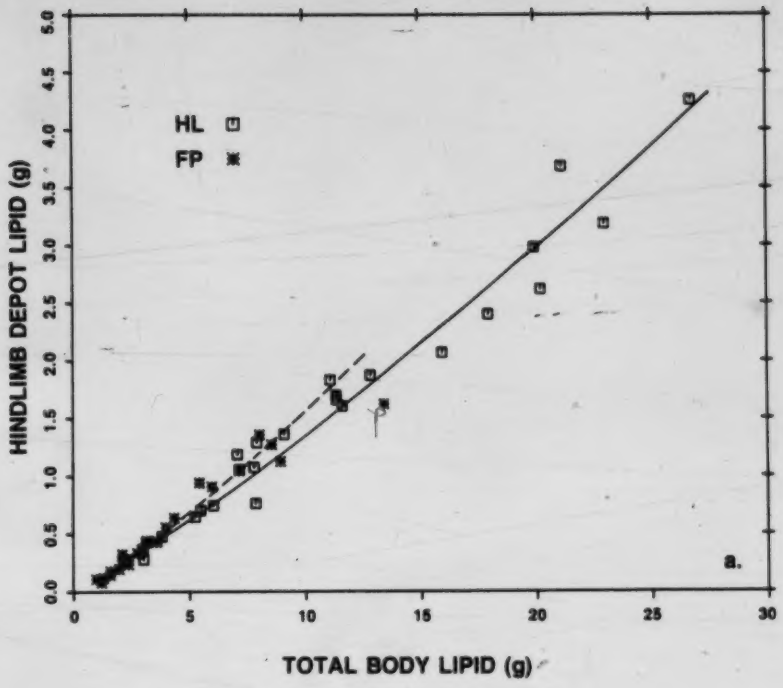
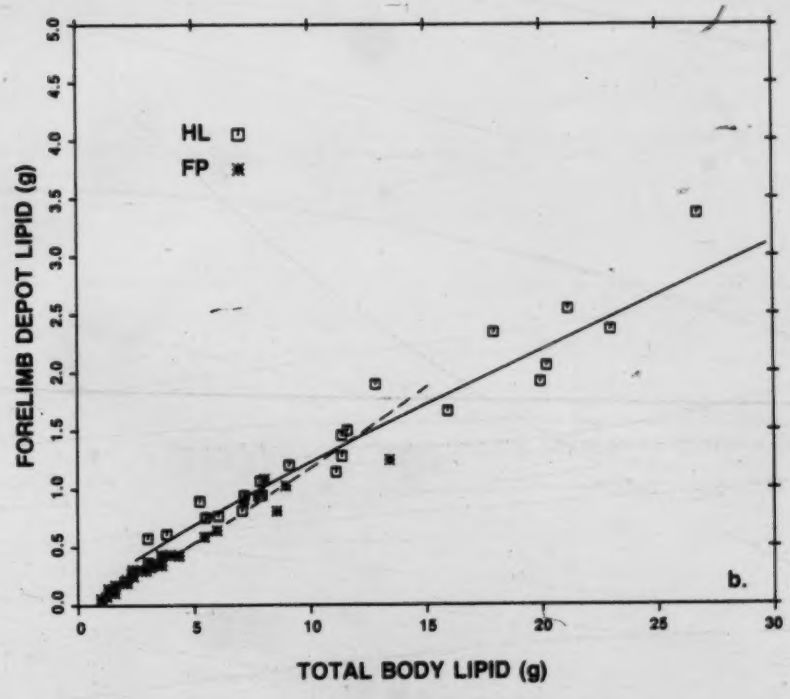
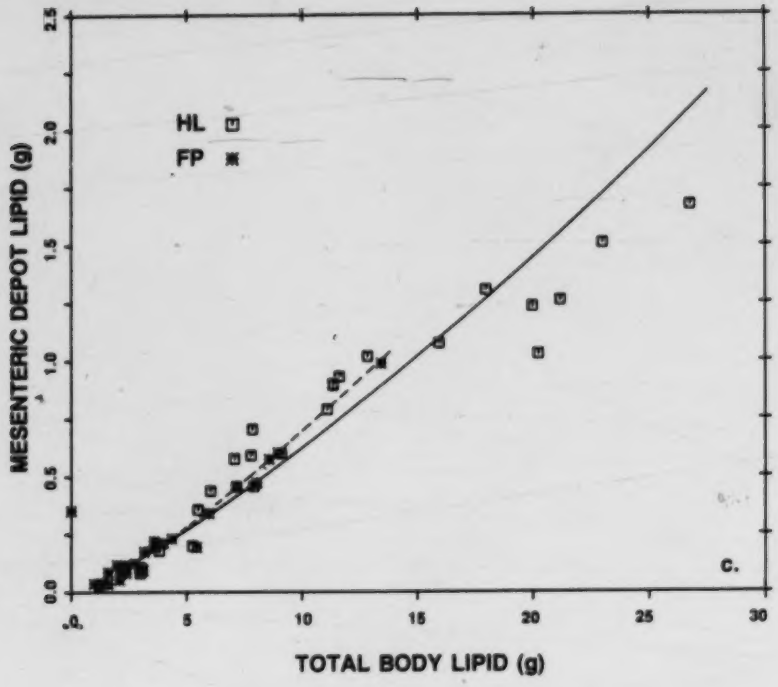
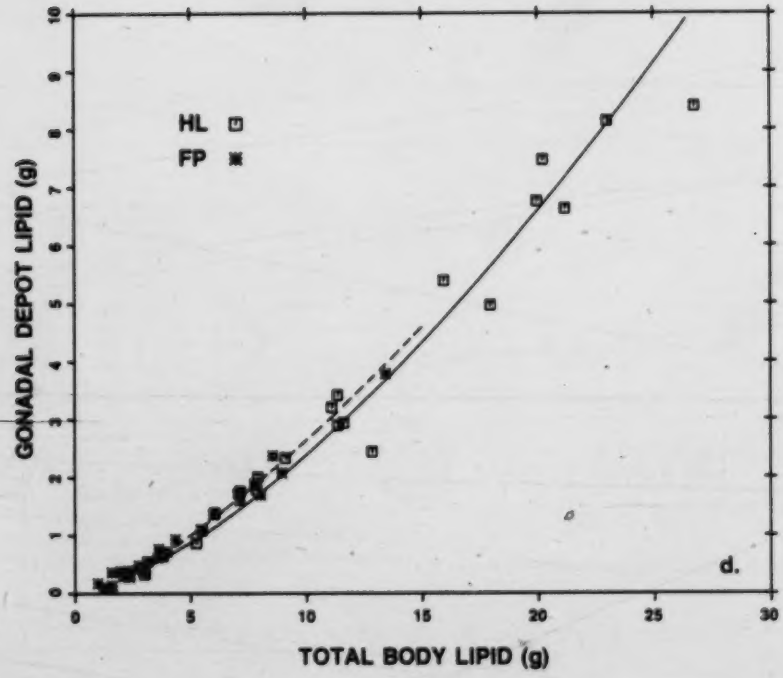

NONADIPOSE TISSUE LIPID (g)
HL
FP
f.
TOTAL BODY LIPID (g)

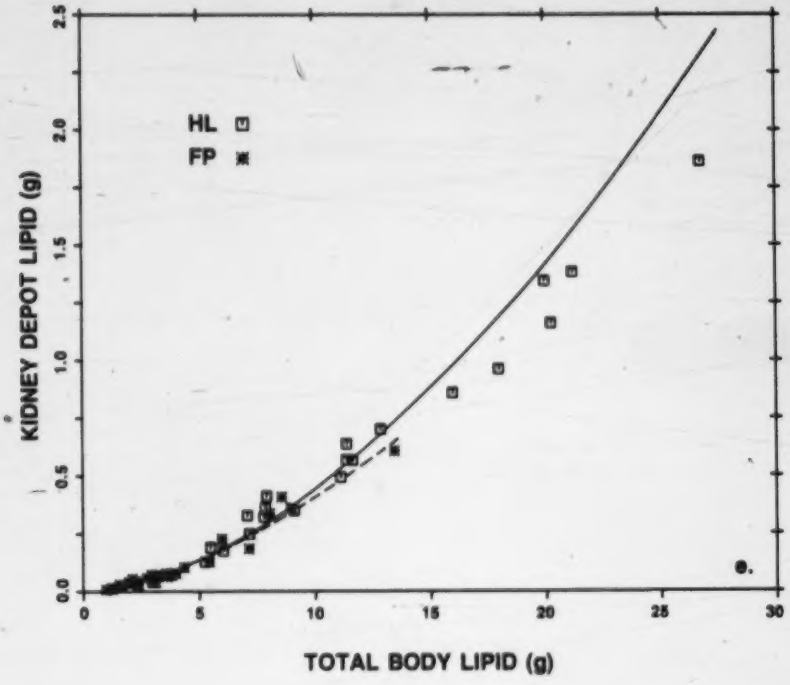
Figure IV.6(a-f) Depot lipid weight relative to total body lipid weight in HL and FP mice (Females). The relationship, between the lipid weight of a specific depot and total body lipid weight, was investigated using the allometric equation described in Chapter II. The predicted allometric relationship between these variables is plotted in each scattergram.


HL
FP
HINDLIMB DEPOT LIPID (g)
TOTAL BODY LIPID (g)
a.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
0
5
10
15
20
25
30


HL
FP
FORELIMB DEPOT LIPID (g)
TOTAL BODY LIPID (g)
b.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
0
5
10
15
20
25
30


MESENTERIC DEPOT LIPID (g)
HL
FP
c.
TOTAL BODY LIPID (g)


GONADAL DEPOT LIPID (g)
HL
FP
d.
TOTAL BODY LIPID (g)


KIDNEY DEPOT LIPID (g)
TOTAL BODY LIPID (g)
HL
FP
e.

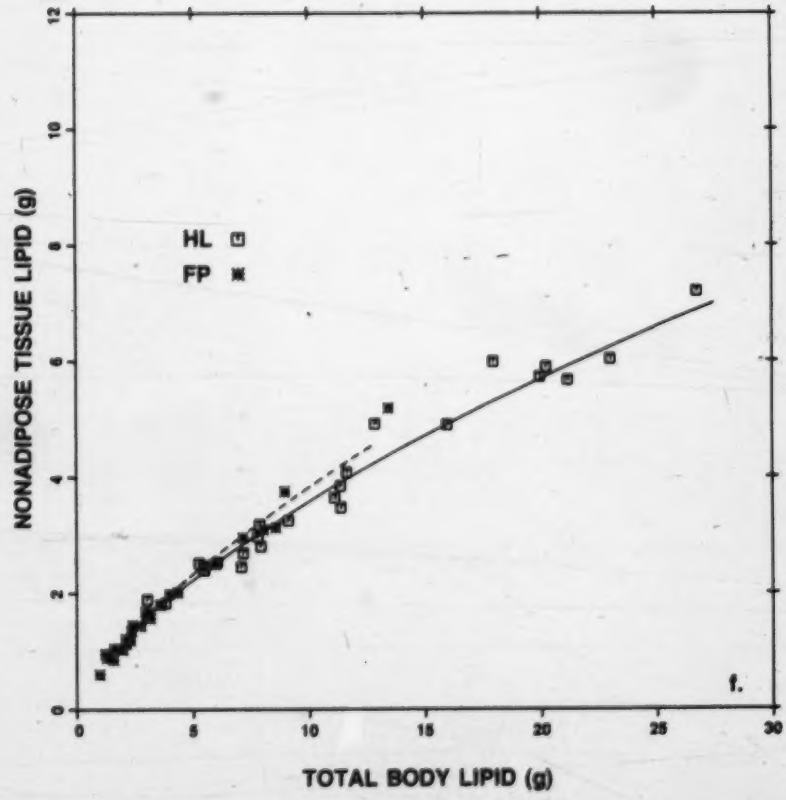
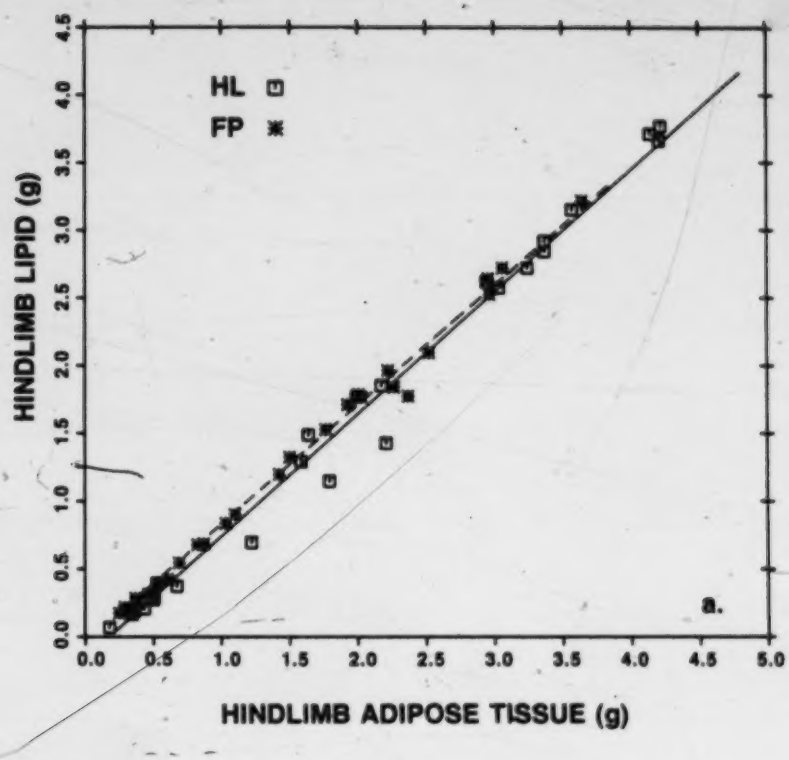
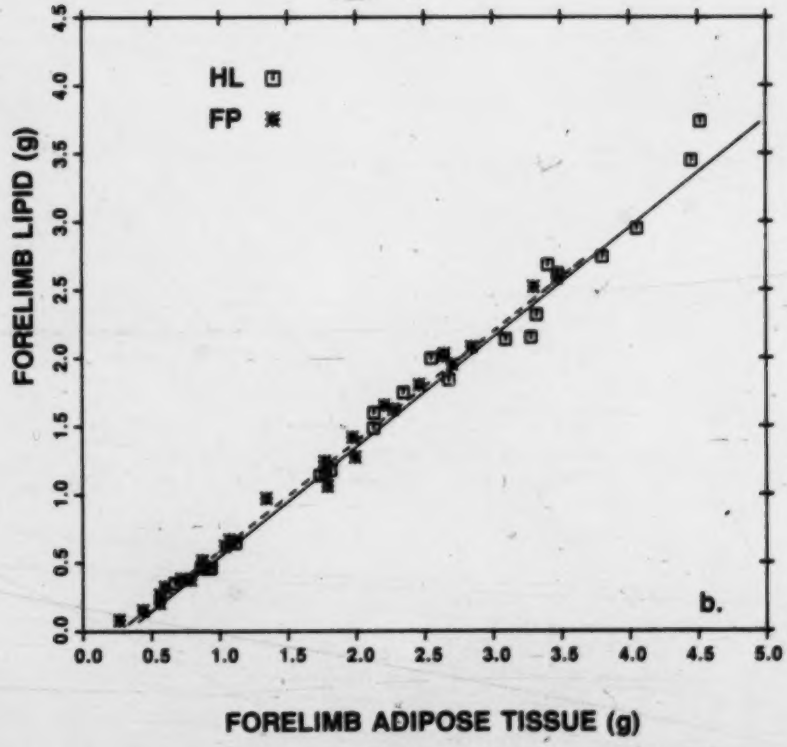
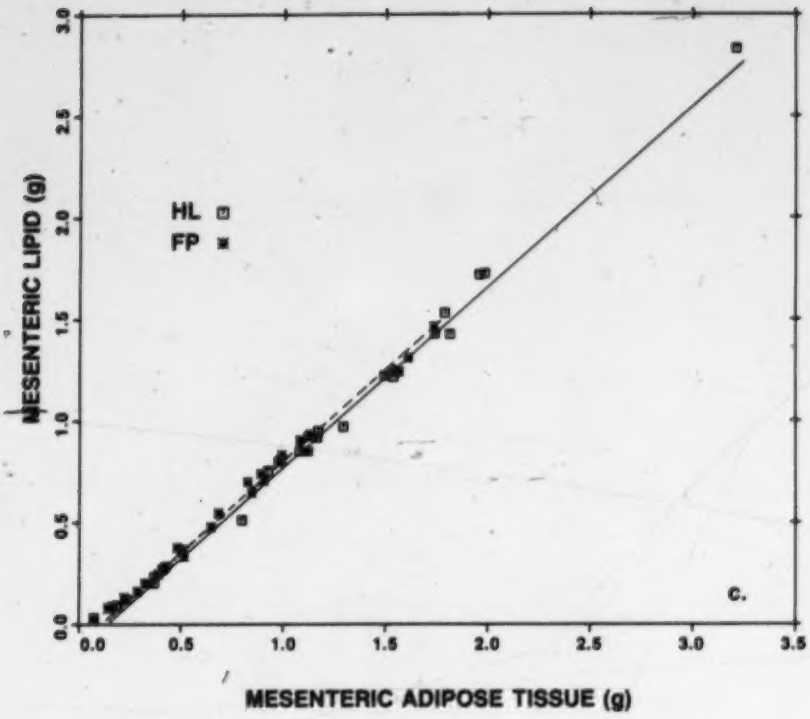
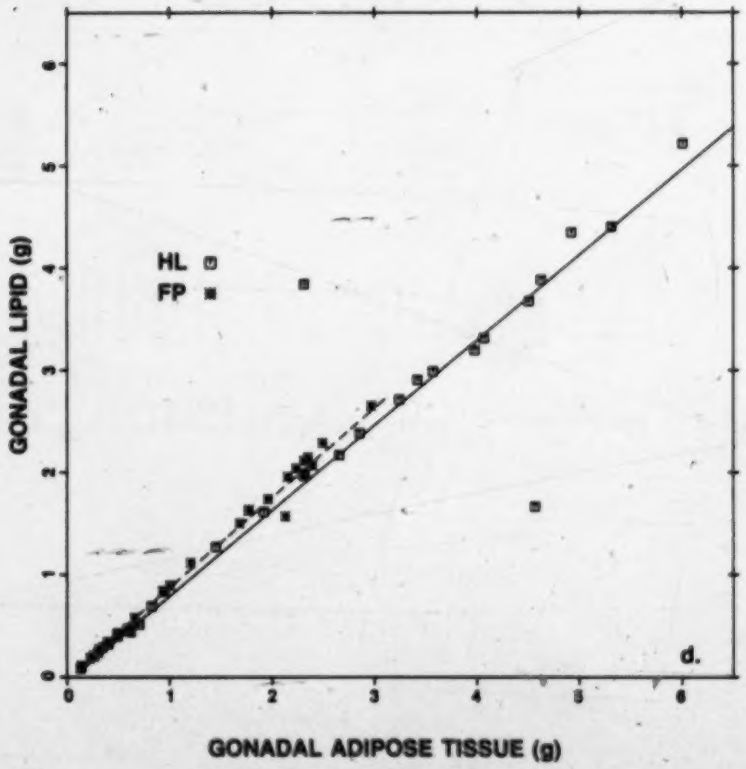

NONADIPOSE TISSUE LIPID (g)
TOTAL BODY LIPID (g)
HL
FP
f.

Figure IV.7(a-e) Depot lipid weight relative to depot adipose tissue weight in HL and FP mice (Males). To examine the pattern of lipid accumulation within each specific depot, depot lipid was plotted relative to depot adipose tissue. The resulting scattergrams revealed a strong linear relationship between these two variables. Linear regressions of depot lipid and depot adipose tissue were, therefore, calculated for each specific depot. The predicted linear relationships, between these two variables, is plotted in each scattergram.


HL
FP
HINDLIMB LIPID (g)
HINDLIMB ADIPOSE TISSUE (g)
a.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0


HL
FP
FORELIMB LIPID (g)
FORELIMB ADIPOSE TISSUE (g)
b.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0


MESENTERIC LIPID (g)
HL
FP
c.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
MESENTERIC ADIPOSE TISSUE (g)


GONADAL LIPID (g)
HL
FP
d.
0
1
2
3
4
5
6
GONADAL ADIPOSE TISSUE (g)

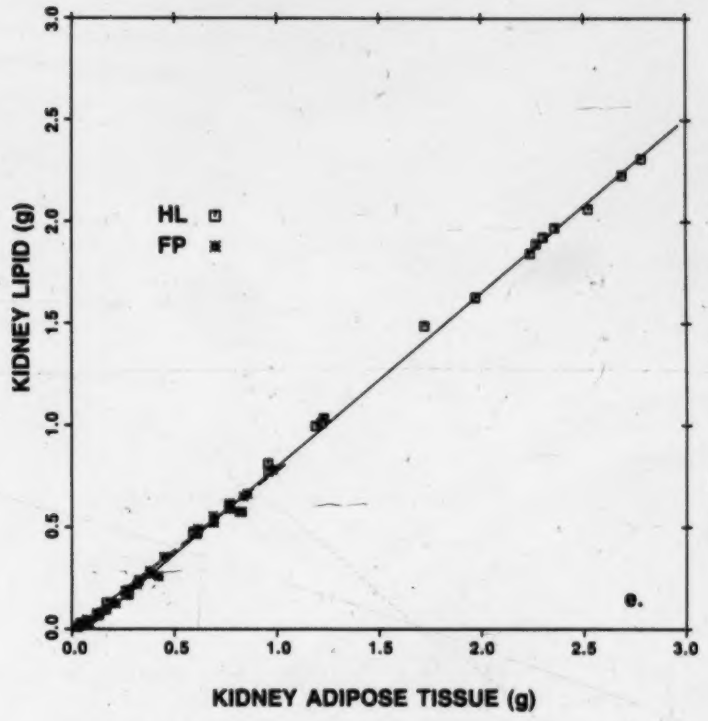
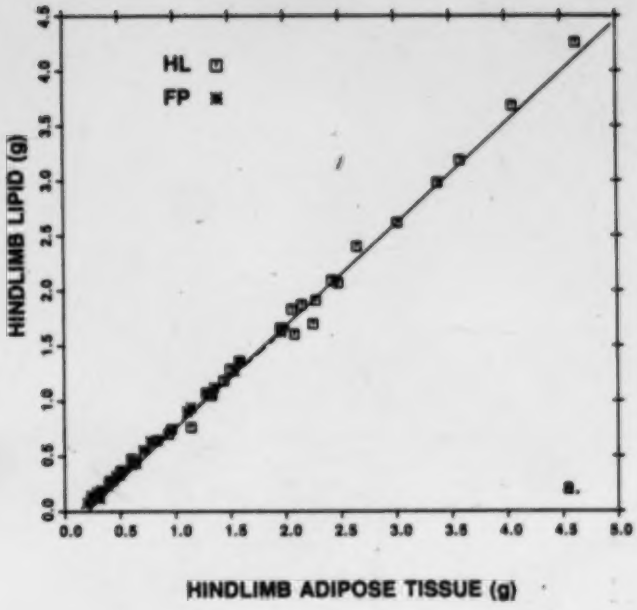
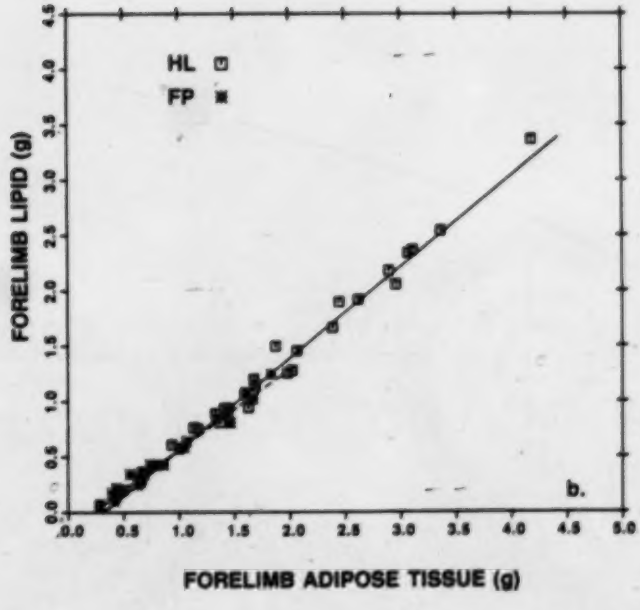
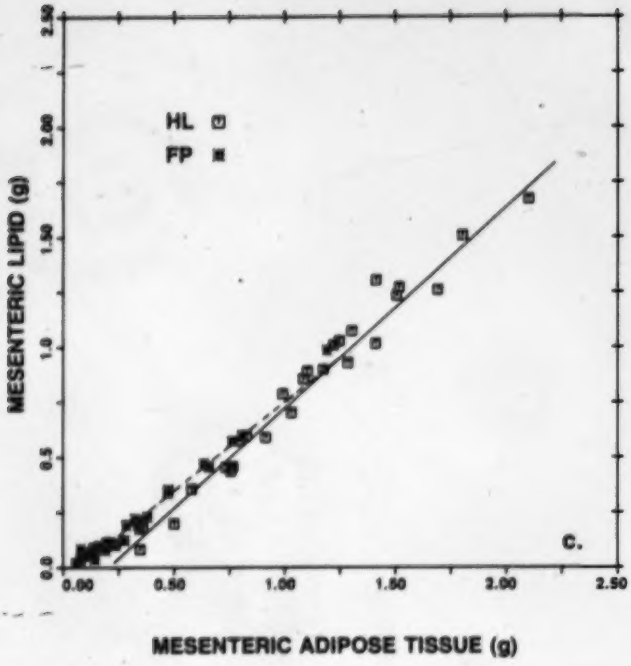
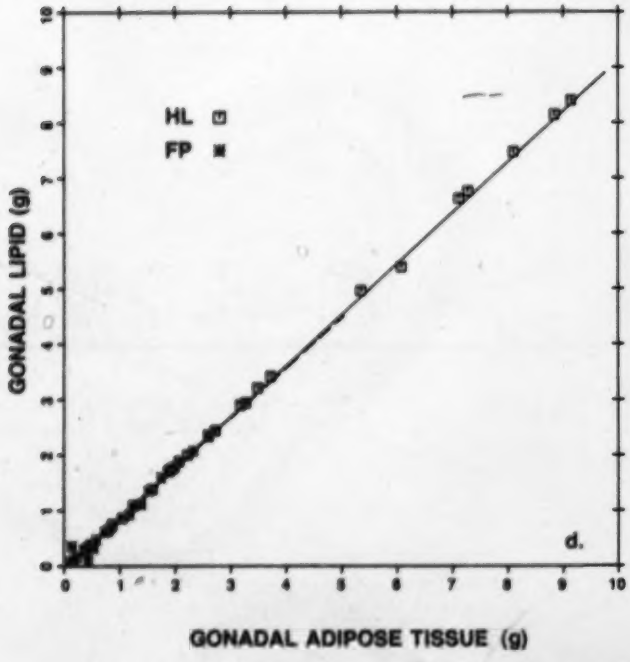

KIDNEY LIPID (g)
KIDNEY ADIPOSE TISSUE (g)
HL
FP
0.0
0.5
1.0
1.5
2.0
2.5
3.0

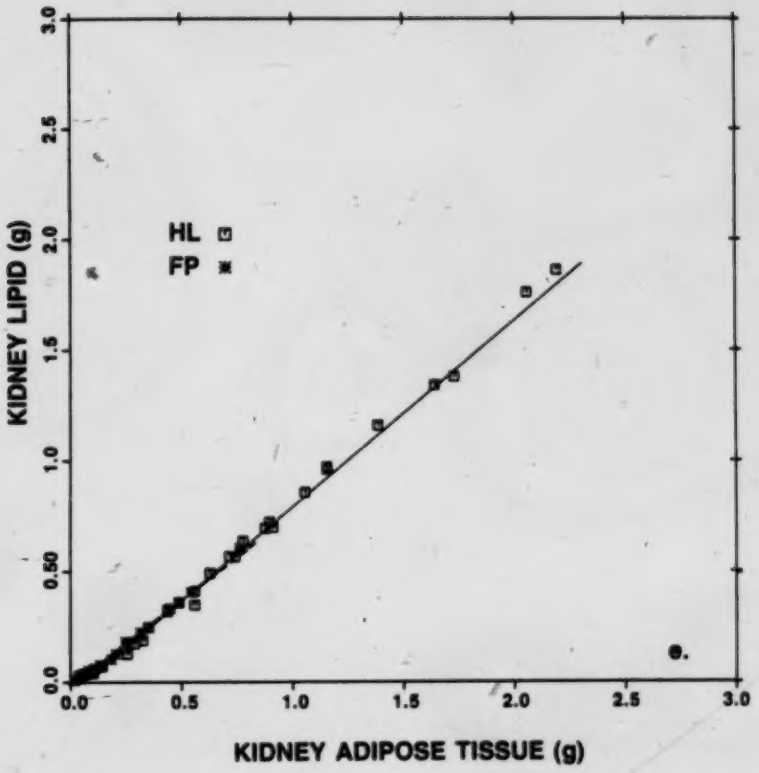
Figure IV.8(a-e) Depot lipid weight relative to depot adipose tissue weight in HL and FP mice (Females). To examine the pattern of lipid accumulation within each specific depot, depot lipid was plotted relative to depot adipose tissue. The resulting scattergrams revealed a strong linear relationship between these two variables. Linear regressions of depot lipid and depot adipose tissue were, therefore, calculated for each specific depot. The predicted linear relationships, between these two variables, is plotted in each scattergram.


HL
FP
HINDLIMB LIPID (g)
HINDLIMB ADIPOSE TISSUE (g)
a.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0


HL
FP
FORELIMB LIPID (g)
FORELIMB ADIPOSE TISSUE (g)
b.
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0


HL
FP
MESENTERIC LIPID (g)
MESENTERIC ADIPOSE TISSUE (g)
c.
0.00
0.50
1.00
1.50
2.00
2.50


HL
FP
GONADAL LIPID (g)
GONADAL ADIPOSE TISSUE (g)
d.
0
1
2
3
4
5
6
7
8
9
10


KIDNEY LIPID (g)
KIDNEY ADIPOSE TISSUE (g)
HL
FP
0.0
0.50
1.0
1.5
2.0
2.5
3.0
e.

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V. IDENTIFICATION OF A VARIANT GROWTH HORMONE HAPLOTYPE IN MICE SELECTED FOR HIGH BODY WEIGHT

A. INTRODUCTION

Animal geneticists have long sought accurate biochemical indicators of an animal's production potential. Previously, researchers attempted to correlate various blood serum protein polymorphisms with economically important traits including growth rate and body size (Neimann-Sorensen and Robertson, 1961; Stansfield et al., 1964; Smith, 1967). The unreliability of such correlations (Stansfield et al., 1964; Smith, 1967) illustrates the need to seek biochemical indicators with an established physiological involvement in the manifestation of the production character. Since several of the major genes regulating body growth are known (Schuler and First, 1985), molecular techniques can be employed in an attempt to identify naturally occurring variant genes which make a positive contribution to growth. The frequency of such variants should be increased by artificial selection for growth-related parameters. Molecular analysis of growth-selected populations should, thereby, aid in the identification of major variants. The identification of such variants will aid in the selection of replacement animals for use in traditional breeding programs. More importantly, the introduction of such variant genes via gene transfer, into populations lacking these variants, could significantly accelerate the rate of animal improvement. To investigate the feasibility of this novel approach to animal improvement, the study reported here employed Southern analysis of genomic DNA, from 'high-growth' and 'non-growth' selected mice, to investigate the hypothesis that the selection pressure could have identified, and acted upon, natural variation at the growth hormone (GH) locus.

B. MATERIALS AND METHODS

Mouse Lines

The history and maintenance of the FP and HL mice employed in this study were described previously in Chapter II.

Logistic Growth Patterns of HL and FP Mice

Mice were weaned at 21 days of age. Male and female mice were caged separately (4/cage). Fifteen mice of each sex were individually weighed every 3rd day from 21 to 84 days of age. The logistic function was fitted to the weight-age data of each individual mouse by a generalized least squares non-linear estimation procedure. The logistic function for the nth mouse is represented by,

$$Y_n(t) = A_n(1-b_n e^{-k_n t})^{-1}$$

where $Y_n(t)$ = body weight (g) at time t (d), b_n = integration constant, k_n = rate at which logarithmic function of weight changes linearly per unit of time (maturing rate) and A_n = asymptotic (mature) weight. Other traits derived from this function were age (t_n^*) and weight (y_n) at the point of inflection, age at maturity ($t_n 0.99$) and mean absolute growth rate (v_n). Each of the estimated parameters of the growth function was analyzed separately using a generalized least squares procedure. The following statistical model was assumed for this analysis,

$$Y_{ijk} = \mu + L_i + S_j + (LS)_{ij} + e_{ijk}$$

where Y_{ijk} = observation on the kth mouse of the ijth subclass, μ = population mean, L_i = effect of the ith genetic line (i=1,2), S_j = effect of the jth sex (j=1,2), $(LS)_{ij}$ = interaction effect of the ith line and the jth sex and e_{ijk} = random effect term.

Restriction Site Analysis of GH Gene

Genomic DNA was prepared from liver tissue according to a modification of the procedure described by Maniatis et al., 1982. The liver tissue was first frozen in liquid nitrogen and then ground to a fine powder with a mortar and pestle. This ground tissue was suspended in 10 volumes of a solution consisting of .5M EDTA (pH 8.0), 100ug/ml proteinase K and .5% Sarcosyl and then placed in a 50°C water bath for 3 hours (suspension was swirled periodically). Following the 3 hour incubation, the DNA was gently extracted 3 times with equal volumes of phenol and then dialyzed extensively against a solution of 50mM Tris (pH 8.0), 10mM EDTA and 10mM NaCl. The dilute DNA solution was then treated with DNase-free RNA (10ug/ml) for 20 minutes, extracted twice with equal volumes of phenol:chloroform and then dialyzed extensively against 10mM Tris (pH 8.0) and 1mM EDTA (pH 8.0). The DNA was stored at 4°C.

For the restriction site analysis, 10ug of genomic DNA/digest was digested with the appropriate restriction endonuclease and fractionated by electrophoresis on .7% - 1.5% agarose gels. Fractionated DNA was transferred to GeneScreenPlus membranes (NEN Research). Membranes were probed with a 0.8 kb Hind III fragment containing the complete rat GH cDNA (Seeburg et al., 1977). The probe was ^{32}P-labeled by synthesis using random oligonucleotide primers. Hybridization was carried out for 24-48 hrs. at 42°C in 50% formamide, 500 μg/ml sheared, denatured salmon sperm DNA, 1.0 M NaCl, 1.0% Sarcosyl, 50 mM Pipes pH 7.0, 5X Denhardts, 20 μg/ml yeast tRNA and 10% dextran sulphate. Filters were washed twice in 100 ml of 2X SSC (5 min each at room temperature); twice in 200 ml of 1X SSC, 1.0% SDS (30 min each at 65°C); twice in 100 ml of .1X SSC (30 min each at room temperature). Filters were then exposed to Kodak XAR-5 film with intensifying screens (Dupont Cronex Lightening Plus) at -70°C.

The orientation of the GH gene was determined with enzymes known to cut within the mouse GH coding sequence (Linzer and Talamantes, 1985). The resulting GH fragments were identified as 5' or 3' with 5'- and 3'-specific rat GH cDNA probes. The positions of the two

identified internal Pvu II sites are conserved in the human, rat and mouse GH coding sequences (Seeburg et al., 1977; DeNoto et al., 1981; Linzer and Talamantes, 1985). Furthermore, in the rat and human GH genes, the 5' end of the structural gene lies approximately 400 bp upstream from the 5' Pvu II site; the 3' end lies approximately 100 bp downstream from the 3' Pvu II site (Barta et al., 1981; DeNoto et al., 1981). The 5' and 3' ends, of the mouse GH structural gene, have been predicted on the basis of this information.

C. RESULTS AND DISCUSSION

The logistic growth curve analysis of HL and FP mice indicated that selection had directed a significant increase in the mean absolute growth rate (v) of HL mice (Table V.1). As a consequence of this accelerated rate of growth, HL mice were 1.5 times heavier than FP mice by approximately 25 days of age; this weight difference was maintained to maturity (see growth curves in Figure V.1). However, selection had little effect upon the rate of maturation (k), age at point of inflection (t^*) or shape of the growth curve (weight at inflection, y^*, relative to asymptotic weight, A) (Table V.1).

A previous genetic analysis of weight-selected mice (Pidduck and Falconer, 1978) indicated that an increased amount, or activity, of GH was involved in the selection response. Natural variation, in both circulating and pituitary GH levels, has been documented in mice (Yanai and Nagasawa, 1968; Sinha et al., 1974). Furthermore, variant forms of the GH molecule (Lewis, 1984), as well as variation in the processing of the GH primary transcript (DeNoto et al., 1981), have been identified in rats and humans.

Two reports have been unable to identify any GH gene sequence variation between mice exhibiting diverse patterns of growth (Parks et al., 1982; Phillips et al., 1982). However, this study has revealed ***between-line*** restriction fragment length polymorphisms (RFLP's) for each of the seven restriction enzymes employed (Figure V.2). In contrast to this between-line variation, a survey of 50 nonsibs from each line revealed no ***within-line*** RFLP's. Each line, therefore, possesses a unique, fixed GH gene haplotype. The two GH haplotypes are

transmitted as alleles of the GH gene; all progeny from HL x FP matings are heterozygous for the two haplotypes (Figure V.3). Densitometic scanning of the F_1 autoradiographic signals revealed that the HL- and FP-associated signals do not differ in intensity. This result suggests that there has been no amplification of the GH gene copy number during the selection process.

The potential for accelerated growth is, therefore, associated with a specific GH haplotype. The association of a restriction pattern haplotype with a variant phenotype has previously facilitated the molecular understanding of β-globin gene lesions responsible for β-thalassemias (Orkin et al., 1982). As indicated in Figure V.4, the identified polymorphic restriction sites occur in both the 5' and 3' flanking regions. Of particular interest is the sequence variation associated with the 3 polymorphic sites (Hind III, Pvu II, Xmn I) which occur within 0.6 - 1.1 kb of the 5' end of the structural gene. In the rat GH gene, this 5' region includes elements which mediate thyroid regulation (Casanova et al., 1985). Moreover, natural sequence variation in the distal 5' flanking sequences of several other genes has been associated with variation in both gene expression (Muskavitch and Hogness, 1982; Estelle and Hodgetts, 1984) and phenotypic expression (Rotwein et al., 1983).

This study indicates that sequence variation also exists within the structural gene itself. Resolution on high percentage agarose gels revealed that one of the HL Pvu II/Xmn I fragments of the structural gene (indicated by an asterisk in Figure V.4) is approximately 15 bp shorter than the equivalent FP Pvu II/Xmn I fragment. In both the human and the rat GH gene, this Pvu II/Xmn I fragment would include the 3' end of the second exon, all of the second intron and the 5' end of the third exon (Barta et al., 1981; DeNoto et al.. 1981).

According to selection theory, high body weight selection should identify, and act upon, functional variation at the loci of genes affecting growth; alleles, or combinations of alleles, which make a positive contribution to growth will be favored within a line selected for the enhancement of a growth-related parameter. The HL GH haplotype may represent a GH allele which is functionally different from the FP GH allele and has, thereby, reached fixation

as a result of the imposed selection pressure.

Agriculturally important variant alleles undoubtedly exist in many livestock populations. Knowledge of the existence of such alleles will greatly aid the selection of replacement breeding animals. In addition to phenotypic considerations, the selection of replacements could include an evaluation of the specific alleles carried by each animal. Furthermore, gene transfer techniques will eventually allow combinations of important variants to be introduced into a population much more rapidly than traditional breeding procedures. The potential of gene transfer to influence growth rate and body size has been illustrated by the accelerated growth of mice bearing metallothionein-I/growth hormone (MT-I/GH) fusion genes (Palmiter et al., 1982; 1983). This fusion construct effectively disrupts all feedback mechanisms which regulate GH gene expression via the natural GH 5' regulatory elements. Unfortunately, in addition to the correlated acceleration of growth in mice carrying this construct, the disruption of the natural GH regulatory region is also associated with physiological changes which reduce the overall fitness of the transgenic animal: the sexual differentiation of certain liver functions is abnormal (Norstedt and Palmiter, 1984) and the fertility of females is impaired (Hammer et al., 1984). Consequently, while the MT-I/GH construct has already been introduced into two livestock species (Hammer et al., 1985), this disruption of all natural 5' regulation may create a deleterious regulatory variant with limited application in livestock populations. However, since natural growth-enhancing variants evolve as a functional component of the genome, their expression in recipient genomes should not cause the undesirable effects associated with fusion genes. While the growth rates of HL and FP mice differ markedly, the two populations do not differ in their fertility, mortality or litter size (unpublished data). Therefore, naturally occurring variants may offer a superior alternative, to fusion constructs, for the enhancement of animal production characters through gene transfer technology.

Table V.1 Means and standard errors of logistic growth curve parameters.

Parameter	FP	HL
Asymptotic weight (A)	25.05 ± 1.00	38.20 ± 0.95**
Age at maturity ($t_{0.99}$)	81.92 ± 3.88	79.52 ± 3.67
Weight at inflection point (y^*)	12.53 ± 0.50	19.10 ± 0.47**
Age at inflection point (t^*)	25.38 ± 0.64	27.14 ± 0.61
Maturing rate (k)	0.090 ± .006	0.098 ± .006
Mean absolute growth rate (v)	0.369 ± .027	0.602 ± .026**

**FP and HL parameters on the same line are significantly different ($P<0.01$).
[1]The logistic function is detailed in the Materials and Methods.

Figure V.1 Logistic growth curves of HL and FP mice. Mice were weaned at 21 days of age. Male and female mice were caged separately. Fifteen mice of each sex were individually weighed every third day from 21 to 84 days of age. The logistic function was fitted to the weight-age data of each individual mouse by a generalized least squares procedure. The logistic function is detailed in Chapter V - Materials and Methods.

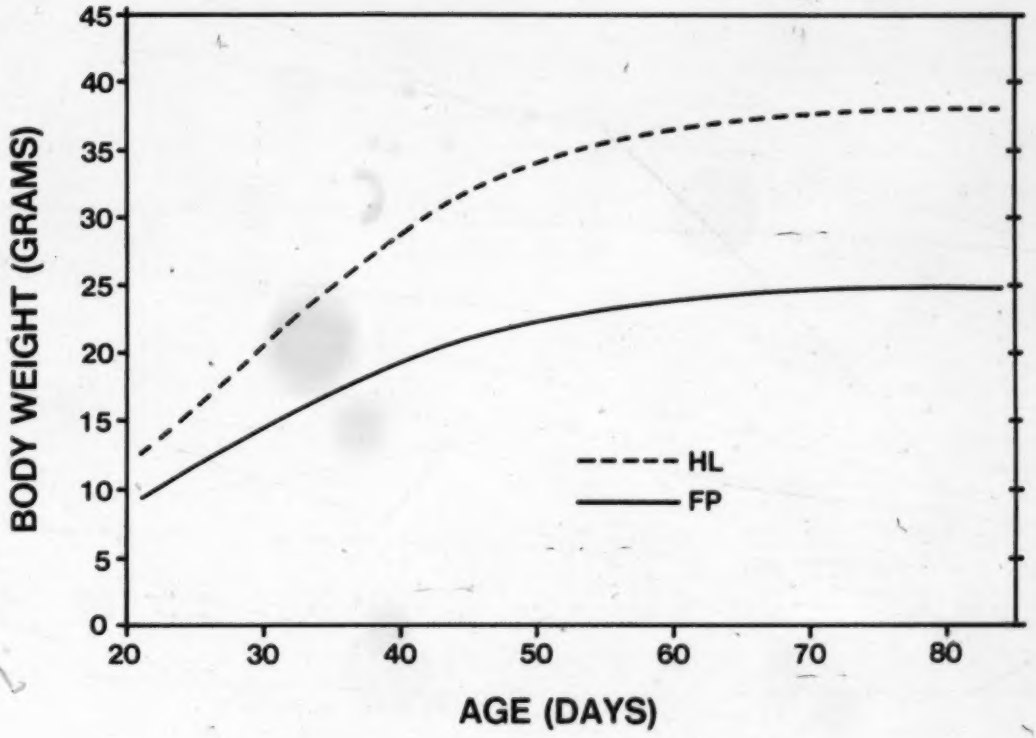

BODY WEIGHT (GRAMS)
45
40
35
30
25
20
15
10
5
0
20
30
40
50
60
70
80
AGE (DAYS)
HL
FP

Figure V.2 Comparison of GH restriction fragment length polymorphisms in HL and FP genomic DNA. For restriction site analysis, 10ug of genomic DNA/digest was digested with the appropriate restriction endonuclease and fractionated by electrophoresis on .7% - 1.5% agarose gels. Fractionated DNA was transferred to GeneScreenPlus membranes. Membranes were probed with a 0.8 kb complete rat GH cDNA. Symbols: E, Eco RI; H, Hind III; B, Bam HI; X, Xba I; Ps, Pst I; Xm, Xmn I; P. Pvu II.

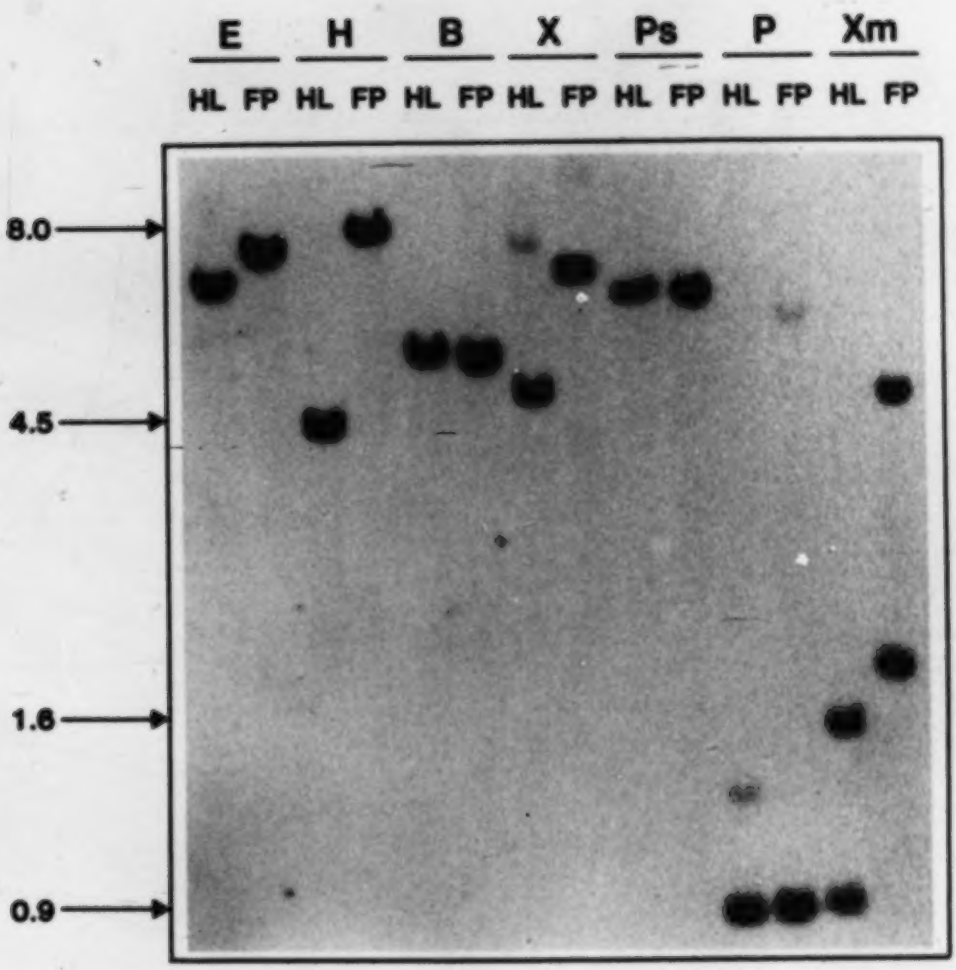

E
H
B
X
Ps
P
Xm
HL FP HL FP HL FP HL FP HL FP HL FP HL FP
8.0
4.5
1.6
0.9

Figure V.3 GH restriction fragments of offspring from HL x FP mating. For restriction site analysis, 10ug of genomic DNA/digest was digested with the appropriate restriction endonuclease and fractionated by electrophoresis on .7% - 1.5% agarose gels. Fractionated DNA was transferred to GeneScreenPlus membranes. Membranes were probed with a 0.8 kb complete rat GH cDNA. Symbols: E, Eco RI; H, Hind III.

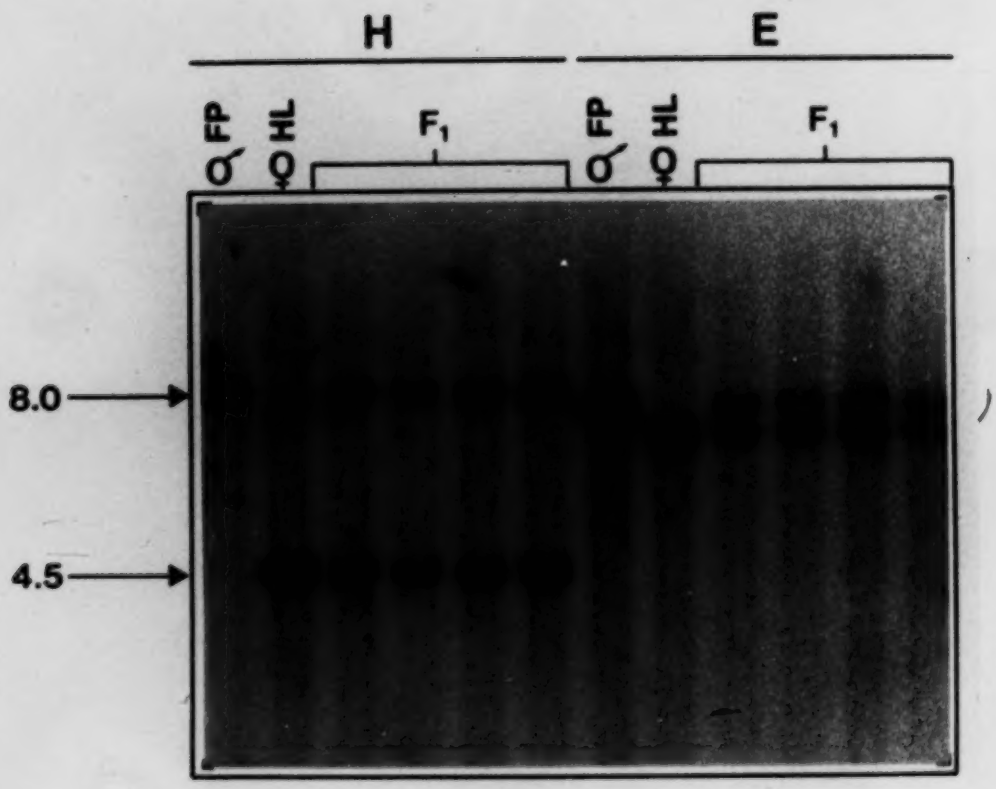
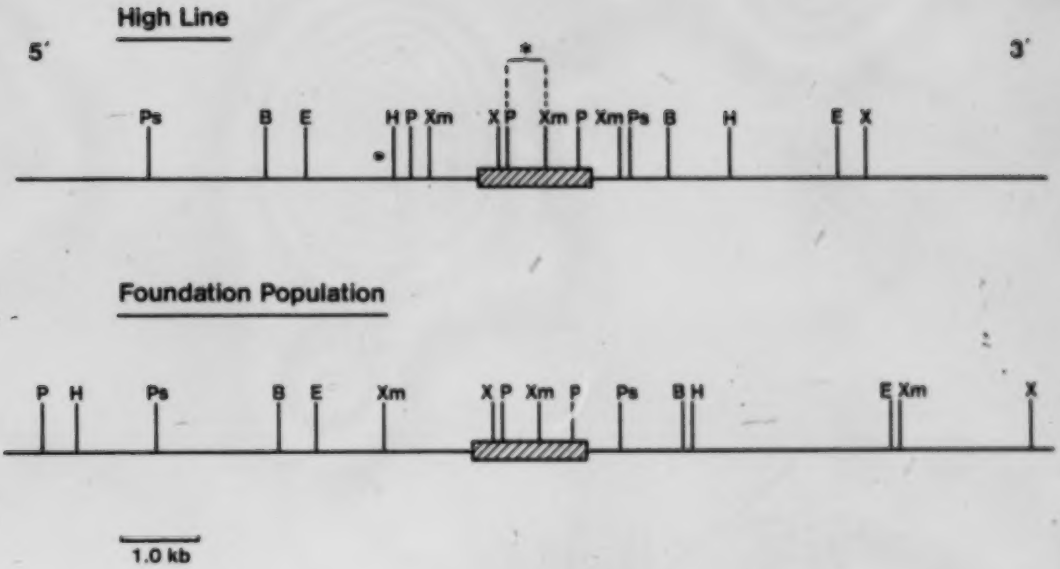

H
E
♂ FP
♀ HL
F1
♂ FP
♀ HL
F1
8.0
4.5

Figure V.4 Map of restriction sites identified by Southern analysis. Restriction fragment denoted with an asterisk is 15 base pairs shorter in HL allele than in FP allele. The orientation of the GH gene was determined with enzymes known to cut within the mouse GH coding sequence. The resulting GH fragments were identified as 5' or 3' with 5'- and 3'-specific rat GH cDNA probes. The positions of the two identified internal Pvu II sites are conserved in the human, rat and mouse GH coding sequences. Furthermore, in the rat and human GH genes, the 5' end of the structural gene lies approximately 100 bp downstream from the 3' Pvu II site. The 5' and 3' ends, of the mouse GH structural gene (cross-hatched box), have been predicted on the basis of this information. Symbols: E, Eco RI; H, Hind III; B, Bam HI; X, Xba I; Ps, Pst I; Xm, Xmn I; P, Pvu II.


High Line
5'
3'
Ps
B
E
H P Xm
X P
Xm P Xm Ps
B
H
E X
Foundation Population
P H
Ps
B E
Xm
X P Xm P
Ps
B H
E Xm
X
1.0 kb

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VI. DEVELOPMENTAL PROFILE OF GROWTH HORMONE mRNA LEVELS IN TWO LINES OF MICE

A. INTRODUCTION

While the age-related developmental profile of pituitary growth hormone (GH) has been characterized in the mouse (Yanai and Nagasawa, 1968; Sinha et al., 1974), there have been no similar reports of the ontogeny of mGH mRNA levels. Since gene expression can be regulated at one (or more) of many levels (Darnell, 1982), characterization of changes in pituitary GH mRNA content may be valuable in identifying the levels of regulation responsible for age- and sex-related variation in pituitary GH levels.

Variations in GH gene expression have also been associated with body growth pattern differences in mice (Pidduck and Falconer, 1978; Cheng et al., 1983). In a previous study to further elucidate the role of the GH gene in the manifestation of divergent growth patterns (Chapter V), a variant GH haplotype was identified within a line of mice selected for high 42 day body weight (High line; HL). Relative to a 'non-growth' selected foundation population (FP), the HL GH haplotype exhibited restriction site polymorphisms within both the structural gene and the immediate 5' flanking region.

To investigate temporal, sex and line variations in mGH gene expression, changes in the pituitary mGH mRNA pool size, throughout the early postnatal growth of HL and FP mice, were therefore characterized.

B. MATERIALS AND METHODS

Mouse Lines

The history and maintenance of the HL and FP mice employed in this study were previously described in Chapter II.

Pituitary RNA Extraction

Six pituitaries were dissected from representatives of each of the 4 line/sex subclasses at 3, 7 and 10 days of age followed by similar samplings at 7 day intervals from 14 to 56 days of age. Upon isolation, pituitaries were frozen in liquid nitrogen, and then stored at -70°C, until RNA extraction.

For extraction of cytoplasmic RNA, 6 pituitaries from each line/sex/age subclass were homogenized in 200 μl of a buffer containing 30 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM KCl, 10 mM $MgSO_4$, 5 mM dithiothreitol, 4 mM EGTA, 5 mM N-ethylmaleimide, 0.5% 2-mercaptoethanol, 25 ug/ml polyvinyl sulphate, 35 ug/ml spermidine and 0.5% (v/v) Triton X-100. The homogenate was then spun at 10,000 rpm for 10 minutes at 4°C. SDS was added to the supernatant to a final concentration of 1%; tubes were inverted several times to ensure mixing of the SDS. This nuclei-free homogenate was then extracted twice with buffer-saturated phenol:chloroform:isoamyl alcohol (25:24:1) and twice with chloroform:isoamyl alcohol (24:1). The RNA was ethanol precipitated from the final aqueous phase overnight at -40°C. The resulting RNA pellet was dissolved in sterile H_2O and was quantified by U.V. absorbance at $O.D._{260}$. This RNA (designated as total pituitary cytoplasmic RNA) was stored at -70°C until assayed.

Northern Analysis

A 2 ug aliquot of total RNA from each line (56 days of age) was added to a denaturation solution (final volume: 20 ul) consisting of 50% (v/v) formamide and 30% (v/v) formaldehyde (37%) in RNA electrophoresis buffer (20 mM EPPS pH 8.0, 10 mM sodium acetate, 0.1 mM EDTA). Samples were then incubated at 65°C for 2 to 5 minutes. RNA was electrophoresed, in 1.5% agarose gels containing 10% formaldehyde, at a constant current of 45 mA for approximately 4 hours. After RNA transfer to nitrocellulose paper (BA85; 0.45 μm), the paper was baked at 80°C for 2 hours in a vacuum oven.

Dot Blot Analysis

Aliquots (0.3 ug) of total RNA from each line/sex/age subclass were blotted onto prewetted nitrocellulose filter paper mounted in a 48 hole microsample vacuum-filtration apparatus (Tyler Research Corp.). Filters were then baked at 80°C for 2 hours in a vacuum oven.

Labelling of Mouse GH cDNA Probe

The GH cDNA (Harpold et al., 1978) was generously provided by Dr. Ronald Evans (Salk Institute) and was ^{32}P-labelled by synthesis using random oligonucleotide primers.

Hybridization of Dot Blots and Northern Blots

Filters were prehybridized (overnight; 42°C) in heat-sealed polyethylene bags with prehybridization buffer containing 50% formamide, 500 ug/ml sheared, denatured salmon sperm DNA, 1.0 M NaCl, 1.0% Sarcosyl, 50 mM Pipes pH 7.0, 5X Denhardts and 20 ug/ml yeast tRNA.

Hybridization was carried out for 24-48 hours at 42°C in prehybridization buffer which included 10% (w/v) dextran sulphate and heat denatured (^{32}P) GH cDNA.

Filters were washed twice in 100 ml of 2X SSC (5 min each at room temperature); twice in 200 ml of 1X SSC, 1.0% SDS (30 min each at 65°C); twice in 100 ml of 0.1X SSC (30 min each at room temperature). Filters were then exposed to Kodak XAR-5 film with intensifying screens (Dupont Cronex Lightening Plus) at -70°C. The autoradiographic dots were quantitated via densiotometry.

C. RESULTS

Pituitary RNA Content

The age-related changes in pituitary cytoplasmic RNA content are presented in Figure VI.1. The profile of RNA levels was similar up to 42 days of age in all four line/sex subclasses. Following this age, HL male, FP male and FP female pituitary RNA levels appeared to plateau or decrease while HL female levels continued to increase up to at least 56 days of age.

Size of Pituitary GH mRNA

Northern analysis of pituitary cytoplasmic RNA revealed a single GH RNA species of 900 base pairs (Figure VI.2.). There was no indication of line or sex variation in the length of this transcript.

GH mRNA Relative to Total Cytoplasmic RNA

In all line/sex subclasses, the age-related changes in GH cytoplasmic mRNA levels, relative to total cytoplasmic RNA, followed a similar pattern (Figure VI.3.). At 7-10 days of age, the GH mRNA concentrations rose dramatically to achieve maximal levels by approximately 21 days of age. After this rapid rise, GH mRNA levels appeared to plateau and then begin a decline to 56 days of age.

While GH RNA concentrations began to rise at the same age in both sexes, males achieved and maintained maximal GH mRNA concentrations that were approximately 1.5-fold greater than those of females. Moreover, males maintained these higher GH mRNA concentrations throughout the duration of the study.

Concentrations began to rise at a younger age, and achieved maximal levels earlier (21 days), in FP males than in HL males. HL male GH mRNA concentrations underwent a more gradual increase, but continued increasing until 42 days of age; at this age, HL male concentrations exceeded those of FP males. HL males maintained these elevated levels to 56 days of age. Within females, the HL and FP GH mRNA concentration profiles were very

similar.

Total GH mRNA Per Pituitary

A graphic representation, of the estimated GH mRNA pool size per pituitary, is presented in Figure VI.4. As illustrated, a clear sex difference existed in the age-related profile of GH mRNA levels; between 35-42 days of age, males exhibited peak GH mRNA levels that were at least 1.5-fold greater than female levels. After achieving these peak levels, male GH mRNA levels decreased to female levels by 56 days of age.

Within both sexes, FP GH mRNA levels appeared higher than HL GH mRNA levels (from animals of the same sex) at most ages up to 35-42 days of age. After this age, HL GH mRNA levels were greater than those of FP mice (of the same sex) and these elevated levels were maintained to 56 days of age.

D. DISCUSSION

The age-related profile of total pituitary RNA presented in Figure VI.1 closely resembles the profile of age-related changes in the anterior pituitary weight of mice described by Yanai and Nagasawa (1968): in males, anterior pituitary weight plateaus between 40 and 60 days of age while anterior pituitary weight in females may continue to increase up to at least 60 days of age. This close correspondence, between the anterior pituitary weight and total pituitary cytoplasmic RNA profiles, likely reflects the large contribution of ribosomal RNA and transfer RNA to the total cytoplasmic RNA pool. Typically, 80-85% of total mammalian cellular RNA is rRNA while another 10-15% consists primarily of tRNA (Maniatis et al., 1982). Since rRNA and tRNA remain at relatively constant levels within the cell (Maniatis et al., 1982), the developmental profile of total pituitary RNA was assumed to be a satisfactory indicator of the age-related ontogeny of pituitary weight via hyperplasia. The use of total pituitary RNA, as an indicator of changes in pituitary weight, was employed to reduce handling of the pituitaries prior to RNA extraction, thereby reducing the risk of

excessive RNA degradation.

As indicated in Figure VI.2, the cytoplasmic RNA isolated from the mouse pituitaries contained a single 900 bp GH RNA species. A GH RNA species of this size has previously been reported as the mature mGH transcript (Linzer and Talamantes, 1985); the RNA species identified by the rGH probe in the dot hybridization analysis was, therefore, assumed to be the mature GH mRNA.

As shown in Figure VI.4, the total pituitary GH mRNA pool size undergoes a tremendous increase between 10 and 35-42 days of age. This increase in pool size probably reflects both an increase in GH-producing cell number (as a result of pituitary hyperplasia) as well as an increase in the pool size per cell. Since anterior pituitary growth continues beyond 35-42 days of age (Yanai and Nagasawa, 1968; Figure VI.1), yet total pituitary GH mRNA content decreases, the GH mRNA pool size per cell probably begins to decrease beyond 42 days of age.

Between 10 and 21-35 days of age, the GH mRNA pool increases faster than the increase in the cytoplasmic RNA pool (Figure VI.3). This increase in the GH mRNA/total RNA ratio likely reflects an increase in the cytoplasmic GH mRNA pool size per cell. Since precursor RNA's (hnRNA) have a very short half-life (Darnell, 1982), the observed GH mRNA increase may reflect an increase in GH gene transcription rather than enhanced processing of a previously unavailable precursor pool. Moreover, since the densitometric data obtained in this study reflect only the cytoplasmic GH RNA pool size (rather than the dynamics of the pool), the age-related increase in pool size could also reflect a decrease in the pool turnover rate. The data presented in Figure VI.3 further indicate that the GH mRNA/total RNA ratio begins to decline after approximately 42 days of age. This decline supports the earlier suggestion that the GH mRNA pool size per cell may begin to decrease following this age.

Changes in the cellular GH mRNA pool size reflect some form of gene regulation. The involvement of thyroid and glucocorticoid hormones in these changes is very probable;

both of these hormones are capable of enhancing GH gene transcription while glucocorticoids may also influence the stability of the GH mRNA (Eberhardt et al., 1982).

The GH mRNA developmental profiles described in this study are very compatible with previous studies of pituitary GH profiles in mice. First evident at day 16 of fetal growth (Slabaugh et al., 1982), pituitary GH content rises until approximately 60 days of age (Yanai and Nagasawa, 1968; Sinha et al., 1974); at this age, GH levels per pituitary may begin to decrease (Yanai and Nagasawa, 1968) or remain stable (Sinha et al., 1974). Notably, the decrease in GH mRNA levels described here begins earlier, and appears to be more dramatic, than the decrease in GH levels reported by Yanai and Nagasawa (1968). In mice, GH levels per unit of pituitary weight increase up to approximately 40 days of age after which levels remain stable. GH mRNA concentrations also increase rapidly during the early postnatal period; however, this increase appears to terminate earlier than the increase in GH concentration. Another notable difference, between the GH mRNA and pituitary GH concentration profiles, is the decrease in GH mRNA concentrations which begins following approximately 42 days of age.

The trend for male mice to achieve and maintain larger GH mRNA pools, than females of the same age, is also similar to the pituitary GH data (Sinha et al., 1974). The sex difference in pituitary GH content, therefore, may reflect an enhanced level of GH gene expression in males (or a decreased rate of GH mRNA turnover) rather than a superior translatability of the GH mRNA present in males.

Within both sexes, the total pituitary GH mRNA pool size began to decrease earlier in FP mice than in the HL mice (Figure VI.3). However, the biological significance of this observation is unclear. Thus, there appears to be little association between GH mRNA pool size and the line-specific growth patterns described in Chapters II, III and V. This observation does not, however, rule out the potential involvement of the GH gene in the growth pattern differences. While there is no apparent size differences between HL and FP GH mRNA transcripts (Figure VI.2), Chapter V described sequence variation between the HL and FP GH

structural genes; the influence of this variation at the RNA and protein levels requires further examination. Furthermore, while this study indicated that FP and HL mice (of the same sex and age) do not differ greatly in their GH mRNA pool size, age-related variation in GH mRNA species remains an unexplored question. To date, only a single mature GH transcript has been identified in mice (Cheng, 1983; Linzer and Talamantes, 1985: Figure VI.2). However, two mature GH transcripts have been identified in cultured rat anterior pituitary cells (Eberhardt et al., 1982). The relative proportions of these two mature transcripts, which differ in the length of the poly(A) tail, can be specifically altered by treatment with thyroid and glucocorticoid hormones (known regulators of GH gene expression (Nyborg et al., 1984)) as well as by the growth conditions of the cells; the larger rGH mRNA predominates during the logarithmic growth phase yet the smaller mRNA gains predominance when the cells become confluent.

The study reported here, therefore, describes an age-related developmental profile of the mouse GH mRNA pool which is similar to previously reported profiles of pituitary GH levels. While both sexes exhibit similar GH mRNA profiles, males achieve larger GH RNA pool sizes than females. However, despite the strong association between the HL GH haplotype and rapid growth (Chapter V), this study revealed little evidence that the involvement of the HL GH haplotype is mediated via the enhancement of the GH mRNA pool size in HL mice.

Figure VI.1 Age-related profile of total pituitary cytoplasmic RNA in HL and FP mice. Six pituitaries were dissected from representatives of each of the four line/sex subclasses at 3, 7 and 10 days of age followed by similar samplings at 7 day intervals from 14 to 56 days of age. Cytoplasmic RNA was extracted according to the the procedure outlined in the Materials and Methods of Chapter VI.

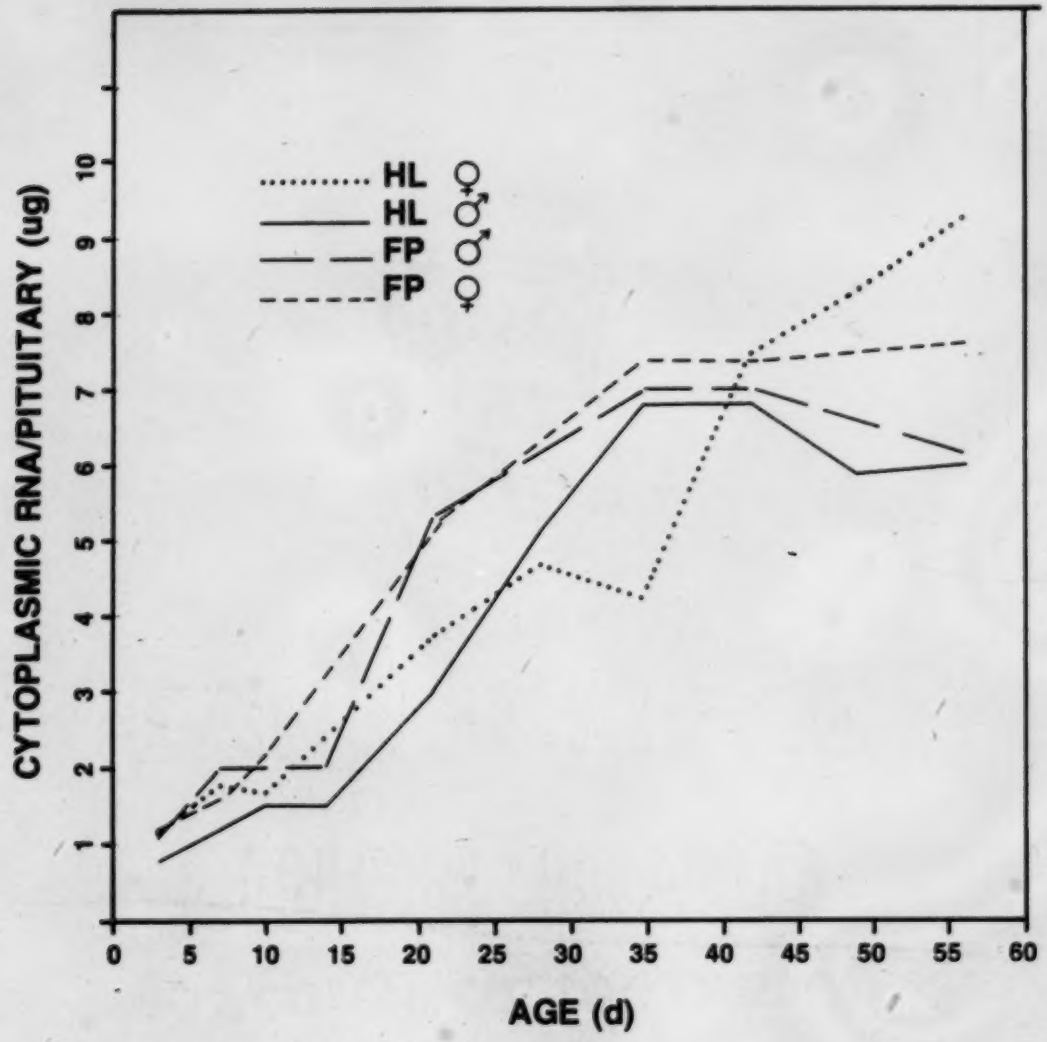
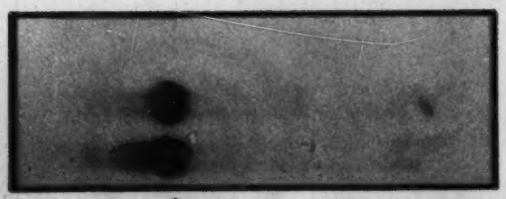

HL ♀
HL ♀♂
FP ♀♂
FP ♀
CYTOPLASMIC RNA/PITUITARY (ug)
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30
35
40
45
50
55
60
AGE (d)

Figure VI.2 Northern analysis of cytoplasmic GH RNA. A 2 ug aliquot of total RNA from each line was electrophoresed, in 1.5% agarose gels containing 10% formaldehyde, at a constant current of 45 mA for approximately 4 hours. After RNA transfer to nitrocellulose paper, the paper was baked at 80C for 2 hours in vacuum oven. The RNA was then probed with a .8kb rat GH cDNA.



mouse liver RNA
FP pituitary RNA
HL pituitary RNA

900 bp.

Figure VI.3 Age-related profile of GH mRNA/total cytoplasmic RNA in HL and FP mice. Aliquots (.3 ug) of total RNA from each line/sex/age subclass were blotted onto prewetted nitrocellulose filter paper mounted in a 48 hole microsample vacuum-filtration apparatus. Filters were baked at 80C for 2 hours in a vacuum oven. The RNA was then probed with a .8 kb rat GH cDNA. The autoradiographic dots were quantitated via densiotometry.

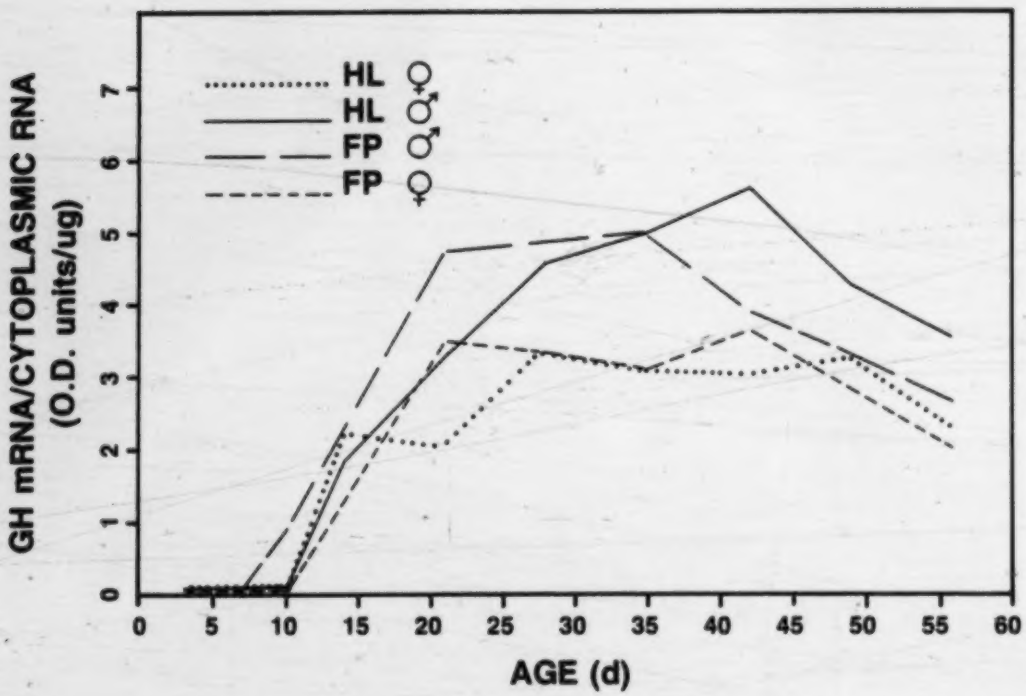
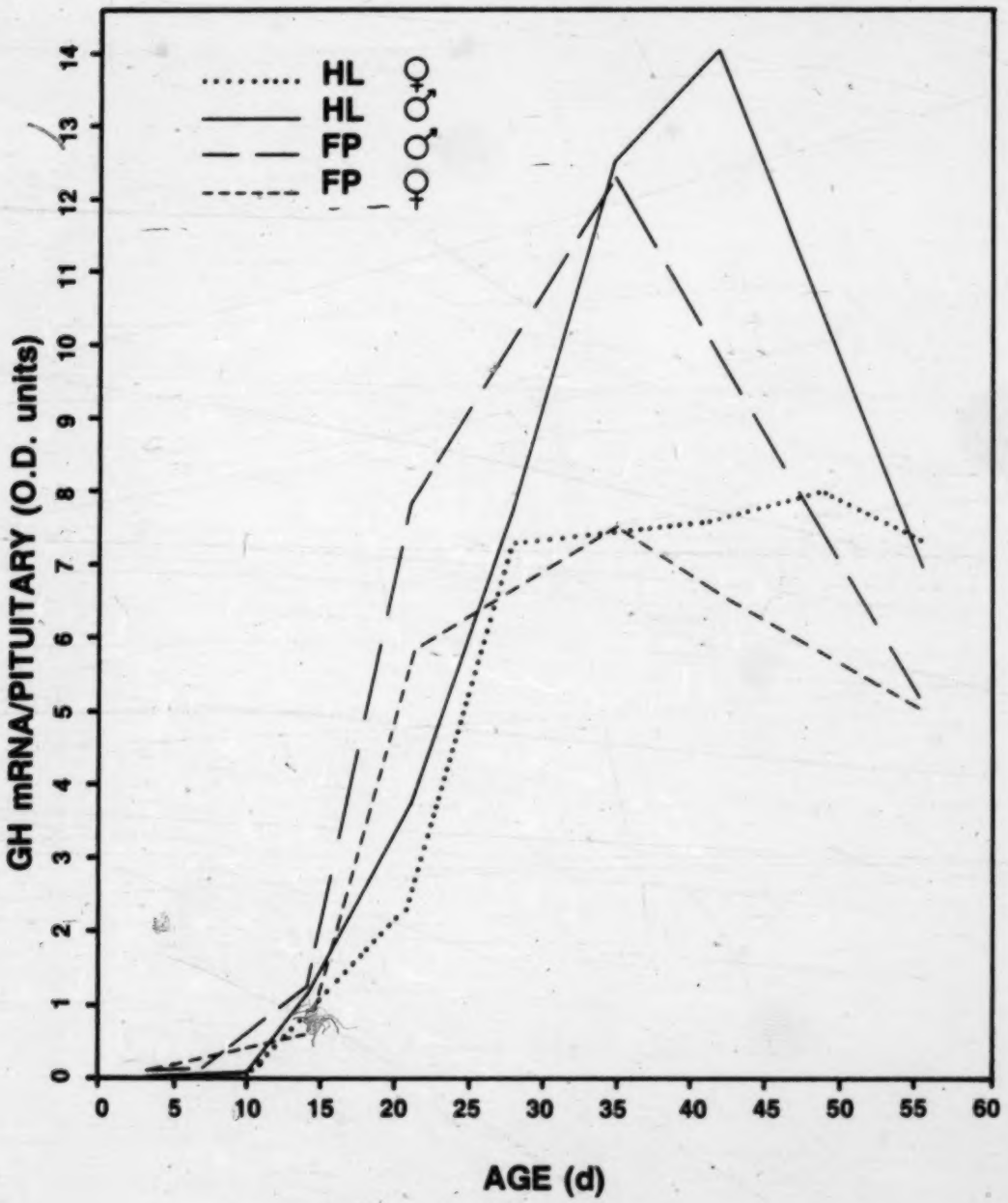

GH mRNA/CYTOPLASMIC RNA
(O.D. units/ug)
HL ♀
HL ♂♀
FP ♂
FP ♀
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30
35
40
45
50
55
60
AGE (d)

Figure VI.4 Age-related profile of GH mRNA/pituitary in HL and FP mice. Aliquots (.3 ug) of total RNA from each line/sex/age subclass were blotted onto prewetted nitrocellulose filter paper mounted in a 48 hole microsample vacuum-filtration apparatus. Filters were baked at 80C for 2 hours in a vacuum oven. The RNA was then probed with a .8 kb rat GH cDNA. The autoradiographic dots were quantitated via densiotometry.


HL ♀
HL ♂♀
FP ♂
FP ♀
GH mRNA/PITUITARY (O.D. units)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
5
10
15
20
25
30
35
40
45
50
55
60
AGE (d)

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VII. GENERAL DISCUSSION

Recombinant DNA technology offers the potential to identify the selection-mediated molecular genetic changes responsible for a characterized phenotypic selection response. The use of a 'high-growth' selection line of mice, to evaluate this potential, offered two conveniences: the phenotypic selection responses of growth-selected mouse lines have been reported extensively in the literature (reviewed by Malik, 1984) - the identified phenotypic selection response of HL mice could therefore be compared with these previously reported responses; in addition, many of the major genes regulating various aspects of mammalian growth have been characterized - thus, once the phenotypic selection response had been characterized, the structure/expression of an appropriate growth-regulating gene could be studied.

The profile of the average 42 day body weight/generation curve of HL mice (Figure II.1) exhibits the general features of the curve characteristic not only of a 'high-growth' selection response (Falconer, 1953; Fowler, 1958) but also of the selection response of most phenotypic characters regulated by 'many' genes (Futuyama, 1979). The gradual increase in body weight, during the early generations of selection, theoretically reflects a phase during which combinations of alleles (which make the greatest contribution to increased body weight) are being organized. Once these combinations have been 'selected', body weight increases/generation decrease and eventually plateau. The data, presented in Figure II.1, therefore suggest that more than one gene was involved in the enhancement of the 42 day body weight of HL mice.

The logistic weight-age curves of HL mice, relative to those of FP mice (Figure II.2, III.1 and V.1), also closely parallel the relationship between the weight-age curves of other 'high-growth' and 'non-growth' selected lines described in the literature (for review see Eisen, 1975). Selection of mouse populations, for the enhancement of a growth-related parameter, consistently accelerates growth rate and elevates mature body size. However, such selection has little effect upon the rate of maturation, age at point of inflection or shape of

the growth curve (Eisen, 1975).

While the direct selection response of HL mice resembled that of other 'high-growth' selection lines, the tissue growth pattern changes were very different. In general, other reported 'high-growth' lines were leaner than 'non-growth' selected control lines prior to the age at which the selection pressure was imposed. Following that age, the 'high-growth' mice grew fatter, relative to body weight, than the 'non-growth' selected mice (for review see Malik, 1984). On the basis of this information, Hayes and McCarthy (1976) suggested that the most probable gene systems involved in a 'high-growth' selection response are those which allow an enhancement of appetite as well as those which allow an increased partitioning of energy into nonfat growth. However, since the increase of nonfat tissue eventually asymptotes, Hayes and McCarthy (1976) further suggested that the selection-directed elevation of energy intake is then available for fat deposition; thus, according to the Hayes/McCarthy model, it is this redirection of energy into fat deposition which causes 'high-growth' selected mice to become fatter than unselected mice.

The relationship between HL and FP patterns of fat deposition, relative to body weight (characterized in Chapters III and IV), appeared to be almost the reverse of those in the previous reports. HL mice were fatter than FP mice, of the same sex and body weight, prior to body weights of 25-30 g. This range of body weights corresponds approximately with an age of 42 days (Figure II.2) - the age at which the selection pressure was imposed. Following this age, HL mice exhibited a lower relative rate of fat deposition than FP mice of the same sex, and thus became relatively leaner than FP mice as body weight increased. Only at fixed body weights, which exceeded the maximum weights achieved by the FP, did HL fat weights exceed those of FP mice.

Therefore, the data presented in Chapters III and IV suggest the need for an expansion of the basic Hayes/McCarthy model of selection-directed compositional changes. While HL mice exhibited an increase in appetite (Bailey, 1985), this increase was associated with a reduced relative rate of fat deposition. Therefore, dependent upon the genetic variation

present in the selection line, 'high-growth' selection appears to be capable of coupling an increased appetite with an enhanced relative rate of nonfat tissue growth. That HL mice exhibited higher lipid weights, at low body weights, may indicate that their elevated appetite exceeded their genetic ability for nonfat growth during an early phase of whole body growth; at heavier body weights, HL mice may be better able to utilize the increased food intake for nonfat tissue growth.

While various environmental factors can influence the partitioning and distribution of adipose tissue, there is clearly a strong genetic component involved in the manifestation of these two characters (Young, 1976). Moreover, the expression of gene systems, which influence these two characters, can be altered by selection for the enhancement of a growth-related parameter: Allen and McCarthy (1980) revealed that individual fat depots were capable of responding differentially to a 'high-growth' selection-mediated change in fat accumulation. However, as described in Chapter IV, the depression of the relative rate of the HL fat accumulation had little influence upon the developmental relationships among the various depots. This limited change in HL fat partition/distribution may reflect the existence of little genetic variability for these characters in the founding animals. Alternatively, the increased growth rate and mature body size of HL mice may have necessitated very little alteration in fat partition/distribution to accommodate any functional changes associated with the acceleration of growth.

On the basis of the phenotypic characterization of HL mice, gene systems which influence body growth rate, mature body size and total adipose/total nonadipose tissue growth patterns were clearly involved in the selection response. The known influences of the GH endocrine unit support the potential involvement of the GH gene (as one of possibly many genes) in mediating this response: GH is not only an important regulator of overall postnatal growth (Wilhelmi, 1982), but has also been demonstrated to enhance both muscle growth (Franchimont and Burger, 1975) and lipid mobilization (Goodman and Grichting, 1983). Furthermore, the GH endocrine system has been suggested to play a role in the regulation of

appetite (Vaccarino et al., 1985).

Restriction endonuclease analysis of the GH gene in HL and FP mice revealed that each line possesed a unique, fixed GH allele. Both of these alleles were very likely present in the foundation gene pool (Salmon et al., 1987) and, thus, did not arise through mutational events which occurred during the selection experiment. Restriction site analysis of an additional three analogous 'high-growth' selection lines, derived from different genetic stock, has revealed only the HL GH allele. Moreover, analysis of an additional five 'non-growth' selected lines (controls of the 'high-growth' lines) has revealed only the FP GH allele in four out of the five lines (Salmon et al., 1987).

The potential for accelerated growth is, therefore, associated with a specific GH allele. According to selection theory, high body weight selection should identify, and act upon, functional variation at the loci of genes affecting growth; alleles, or combinations of alleles, which make a positive contribution to growth will be favored within a line selected for the enhancement of a growth-related parameter. The HL GH allele may represent a GH allele which is functionally different from the FP GH allele and has, thereby, reached fixation as a result of the imposed selection pressure.

According to a simplistic genetic model of selection theory, the absence of the imposed 'high-growth' selection pressure would be predicted to result either in the retention of both GH alleles or in the random fixation of one allele due to genetic drift. Thus, the fixation of the FP allele, within five of six 'non-growth' selected lines, was unexpected. A possible explanation for this phenomenon is that, in the absence of high body weight selection, the FP allele was most compatible with the general fitness of the mice.

Variations in GH gene expression have previously been associated with variations in body growth patterns in mice (Pidduck and Falconer, 1978; Cheng et al., 1983). However, the involvement of a GH allele, in the acceleration of growth, could be manifest at one or more (of many) levels of gene expression (Darnell, 1982). Characterization of the age-related profile of GH mRNA, in HL and FP mice, was thus potentially valuable in understanding the

involvement of the HL GH allele in the accelerated growth of HL mice. This characterization revealed that the involvement of the HL GH allele is probably not mediated via the enhancement of the GH mRNA pool size in HL mice.

Therefore, within the limitations of the technology employed, the results of these studies provided support for the present genetic foundations of selection theory (Clarke, 1975; Mayr, 1978). As is suggested by this theory, direct selection pressure on a phenotypic character appeared to effectively mediate a change in the allelic frequencies of a gene with known involvement in the regulation of the selected character. Furthermore, the identification of only the FP GH allele, within five of six 'non-growth selected mouse lines, provided important information for the future analysis and interpretation of selection responses: the absence of the experimentally imposed selection pressure, within populations used as controls in selection experiments, should not be considered to indicate that the genome is free from all indirect selection pressure. The probable fixation of the FP allele indicates that control populations may, thus, not truly be unselected populations, but may be the subject of a myriad of environmentally imposed selection pressures.

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